DVM1 Clinical Pathology of Liver and Biliary Tract Lecture Notes 2023

Detecting liver disease

Because the liver has a large functional reserve and can regenerate, a hepatic injury must be considerable or chronic and recurrent to cause overt hepatic dysfunction or failure.

Detection depends on

1. a thorough history and physical exam
2. screening clinical pathology tests (haematology, biochemistry profile, urinalysis)
3. specific clinical pathology tests, e.g., functional tests, serology
4. imaging
5. cytology (FNA, abdominal fluid analysis) and/or hepatic biopsy

Clinical findings with liver disease

- vary depending on the type, mechanism, and chronicity of the insult.
- Common signs are anorexia, vomiting, diarrhoea, weight loss, and fever.
- With a severe, diffuse liver injury, animals may become jaundiced and demonstrate polyuria and polydipsia (PU/PD), coagulation abnormalities, and ascites. Ascites develops because of portal hypertension (typically associated with the formation of acquired portosystemic shunts) and concurrent hypoalbuminemia (decreased plasma oncotic pressure).
- Hepatic encephalopathy (HE) develops in:
    - acquired liver disease associated with diffuse fibrosis and portosystemic shunts
    - acute fulminant liver failure
    - secondary to congenital portosystemic shunts (congenital malformations of the portal vein that shunt portal blood directly to the systemic circulation).
- Faeces colour may change with complete occlusion of bile ducts (acholic or pale-coloured faeces) or increased enteric bilirubin elimination (green faecal colour).
- Hepatomegaly:
    - diffuse infiltrative (inflammation, neoplasia) or storage disorders
    - acute extrahepatic bile duct obstruction (EHBDO)
    - congenital biliary cystic malformations
    - passive congestion
- Micro hepatica (small liver) usually reflects:
    - portal venous hypoperfusion
    - diversion of enteric hepatotropic factors usually delivered in the portal circulation (MD, PSVA)
    - presence of chronic hepatic fibrosis in dogs

Clinical Pathology Testing for Liver Disease

Tests for detection of liver disease include:

- Haematology (CBC)
- Routine Biochemistry
    - Indicators of hepatocellular damage – increased leakage/damage enzymes
    - Indicators of cholestasis
        - increased cholestasis enzymes
        - increased bilirubin
    - Indicators of dysfunction - decrease in substances produced by the liver – urea, cholesterol, albumin, glucose, and reduced conjugation of bilirubin
- Liver-specific functional tests – bile acids, ammonia, protein C

- Urinalysis – bilirubinuria, urobilinogen, ammonium biurate crystalluria
- Coagulation testing
- Cytology

Complete Blood Count (CBC)

Depending on the severity and underlying cause of liver disease, nonregenerative or regenerative anaemia may develop. Severe or acute anaemia can impact the liver through hypoxia, causing alterations in hepatocyte membranes, leading to leakage enzymes and induction of ALP.

Altered RBC morphology (**poikilocytes**, irregularly irregular RBCs) is common in cats with cholangiohepatitis and hepatic lipidosis.

Cats with hepatic lipidosis, severe cholangiohepatitis, or extrahepatic bile duct obstruction may develop **Heinz bodies**, reflecting oxidative injury that may lead to haemolysis.

Severe hypophosphatemia in hepatic lipidosis may develop secondary to a re-feeding syndrome and cause haemolysis severe enough to require a blood transfusion; this can be avoided by providing fluid therapy supplemented with potassium phosphate when nutritional support is implemented.

In dogs with diffuse necro-inflammatory liver disease (altered sinusoidal perfusion), RBCs with **microvascular shearing** (e.g., schistocytes, acanthocytes) may be seen.

RBC **microcytosis** is common in congenital or acquired portosystemic shunting. Although the pathologic mechanism remains unclear, it likely relates to functional iron deficiency.

Leukogram findings are variable and can include:

- Leukocytosis may reflect inflammatory, infectious, necrosis, neoplasia, glucocorticoid (endogenous release or administration).
- Leukopenia can reflect sepsis or toxicosis.

Mild thrombocytopenia can develop due to reduced thrombopoietin production by the liver.

Liver Enzymes

Liver disease is often first suspected based on increased liver enzyme activity. However, abnormally increased liver enzyme activity is considerably more common than the prevalence of significant liver disease. A broad spectrum of non-hepatic disorders may influence liver enzyme activity. It is essential to recognize that liver enzyme measurements are not liver function tests but rather reflect hepatocyte membrane integrity, hepatocyte or biliary epithelial necrosis, cholestasis, or induction phenomenon.

The pattern of liver enzyme abnormalities related to the signalment, history, total bilirubin concentration, serum bile acid values, and comorbid conditions/medications provides the first indication of a liver-specific disorder.

A full assessment of liver enzyme aberration considers:

1) The predominant **pattern** of enzyme change (hepatocellular leakage enzymes vs. cholestatic enzymes).

2) The **magnitude** of increase of enzyme activity above the normal reference range

- mild is <3 times the upper reference range
- moderate is 3–9 times
- marked is >10 times

3) The **rate and nature of change** (increase or resolution) with **sequential sampling.** Recognizing whether enzyme abnormalities are persistent or cyclic helps categorize likely causes.

4) **The patient's age**. Age-appropriate reference ranges for serum liver enzyme activity are essential to interpret laboratory values in puppies and kittens. Plasma enzyme activities of ALP and GGT in neonatal dogs and cats are remarkably higher than those of adults. Differences reflect physiologic adaptations during the transition from foetal and neonatal life stages, colostrum ingestion, maturation of metabolic pathways, growth effects, differences in the volume of distribution and body composition, and nutritional intake. Serum activities of ALP, AST, CK, and LDH in neonates usually increase significantly during the first 24 hr of life. In kittens, serum activities of ALP, CK, and LDH exceed adult values through 8 wk. of age. Serum ALP increases remarkably in day-old puppies and kittens after colostrum ingestion, as observed in neonatal calves, lambs, pigs, and foals.

5) **Breed and clinical signs.** Up to 5% of clinically "normal" animals can have borderline abnormal enzyme values. Breeds predisposed to hepatic disease with enzyme abnormalities warrant early investigation, e.g., copper storage hepatopathy in Bedlington's

Serum levels of an enzyme can increase due to:

- cell damage (enzyme leakage)
- enzyme induction (increased synthesis of the enzyme)
- cell proliferation (increased tissue mass, e.g., hepatic neoplasia)
- decreased clearance (e.g., reduced renal excretion)
- increased ingestion/absorption (e.g., colostrum)

.

Decreased enzyme activity is usually of no diagnostic importance, i.e., not clinically significant, but can reflect decreased tissue mass (i.e., micro hepatica), poor sample handling (degradation), inhibitors in the serum (e.g., EDTA), or an inappropriate reference interval.

Note: enzyme activity is **NOT** a measure of FUNCTION

CELL DAMAGE ENZYMES (LEAKAGE ENZYMES)

ALT Alanine aminotransferase

AST Aspartate aminotransferase

GLDH Glutamate dehydrogenase

SDH Sorbitol dehydrogenase

LDH Lactate dehydrogenase

Cell damage leads to enzymes in cytosol or organelles e.g., mitochondria leaking into the extracellular space and then reaching the serum. Enzyme levels often rise within hours of cell injury.

The level of serum enzyme rise depends on

- degree of damage - minor (cell injury) to major (cell death)
- the extent of damage (focal vs. diffuse)
- duration (acute vs. chronic) of hepatocyte damage
- enzyme half-life
- tissue perfusion

The magnitude of the increase in enzymatic activity does not necessarily correlate with clinical manifestations of hepatic insufficiency. It does not indicate how reversible a process is with chronic progressive disease, low numbers of hepatocytes undergoing damage or necrosis at any one time. Activities may be within reference intervals or only mildly increased, despite the presence of hepatic insufficiency. Hepatic atrophy may result in low activity due to a lack of hepatocytes

Injury is often accompanied by cell swelling, inflammation, and necrosis – can alter bile flow resulting in intrahepatic cholestasis and thus concurrent increases in cholestasis parameters

ALT Alanine aminotransferase

- ALT is a **cytoplasmic enzyme** that catalyses a reaction to convert alanine to pyruvate for entry into either the gluconeogenesis pathway or the citric acid cycle.
- Found in hepatocytes and small amounts in muscle
- Hepatocytes of horses, pigs, and cattle have little ALT due to low enzyme activity in liver tissue, and so it is not a useful marker of hepatocyte damage in these species. Any increased ALT activity in these animals, horses, ruminants, pigs, and birds is more likely due to muscle injury.
- Elevations in ALT are not considered liver-specific in rabbits as other tissue injuries can also lead to increases.
- The highest concentrations of ALT occur in dogs' and cats' hepatocytes, so it is most used in these species.
- Marked increases may be seen with acute or severe liver injury, e.g., trauma, necrosis, infectious disease, neoplasia
- Minor increases can occur with marked muscle damage
- In dogs, ALT has a clearance half-life of about 60 hours and less in cats. (24 hrs)
- ALT 3hrs cat, 3d dog
- ALT activity increases within 12 hours of an insult to the liver causing damage, peaks at 48 hrs, and returns to reference range over 2-3 weeks.
- Increased blood glucocorticoid concentrations commonly result in increased serum ALT activity in dogs. The serum ALT activity typically increases 2-5-fold due to steroids – due to a combination of steroid-induced hepatopathy and induction of ALT production.
- Anticonvulsant drugs also cause increased serum ALT activity in dogs, possibly because of hepatocyte injury as well as increased enzyme production (induction), e.g., Phenobarbital, primidone, phenytoin
- Endocrine diseases can also be associated with increased ALT, such as diabetes mellitus, hyperthyroidism.
- Dehydration and cardiac disease may also lead to increases in ALT due to **hypoxic damage** to hepatocytes due to hypovolaemia or congestion.
  (haemolysis in **cats**, pigs only can increase)

AST Aspartate aminotransferase

- AST is found in the **cytoplasm and mitochondria of hepatocytes and myocytes** (skeletal levels are higher than cardiac), erythrocytes, and low levels are also present in the kidney and brain (which don't significantly contribute to serum levels).
- This enzyme catalyzes the conversion of aspartate to oxaloacetate, which can enter the citric acid cycle.
- AST has a shorter half-life in dogs than ALT and thus may give a better indication of active hepatocyte damage. The half-life of AST 7-8 d horses, one day in dogs, 1 hr in cats
- Very useful as a marker of liver disease in cattle, horses, rabbits – however, as AST is in myocytes, elevations may also occur with muscle damage and necrosis, with levels higher seen with myopathies than liver disease, e.g., rhabdomyolysis, recumbent animals, vit E deficiency, infectious myositis, muscular dystrophy. When there is active muscle disease, both AST and CK will be elevated (see CK next).
- Increased AST activity with normal CK activity would suggest that the source of the AST is the liver and that hepatocyte injury has occurred. Increased AST with increased CK activity would suggest that muscle injury has occurred rather than liver damage.
- Increased AST will occur with sample haemolysis

(CK Creatine Kinase)

- **NOT** a liver damage marker- **muscle-specific,** so it helps evaluate the cause of AST and ALT elevation.
- CK has a very short half-life, ~2 hours in horses. Activity increases quickly (peaks at 6-12 hours) and returns to normal within 24-48 hours after acute, transient muscle injury. Persistent or ongoing muscle injury will maintain high CK concentrations. In contrast, AST (which has a longer half-life) will increase more gradually after muscle injury and stays elevated for longer than CK.

GLDH Glutamate dehydrogenase

- Glutamate dehydrogenase is present at high concentrations in the livers of dogs, cats, horses, and ruminants, and at low concentrations or excreted in other tissues (kidney, intestine, muscle) and so is considered a liver-specific enzyme.
- GLDH is mostly located in the mitochondria within the cell, with small amounts associated with microtubules and the ER – hence takes a more severe liver injury to cause release than ALT or AST.
- Centrilobular levels are highest – thus, hypoxia increases GLDH more than ALT or AST
- ALT is considered superior to GLDH for detecting hepatocyte injury in dogs and cats.
- In ruminants and horses, it is more liver-specific than AST. So it is a valuable indicator of acute hepatocellular damage but is not very sensitive for chronic or mild liver disease.

SDH Sorbitol dehydrogenase

- a cytoplasmic enzyme, widely used to assess ruminants and horses for hepatocellular damage in the USA but not Australia
- Sorbitol dehydrogenase can be used in all species, but ALT is superior in cats and dogs
- Labile enzyme - decreases rapidly with storage: very specific if the sample is fresh (< 5hrs at room temp, 24hr 4^{o})

Forms of Cholestasis

Cholestasis may result in hyperbilirubinemia, but sometimes bilirubin levels remain normal. Forms of cholestasis include:

1. **Structural cholestasis**

Structural cholestasis involves a physical impediment (**obstruction**) to bile flow. It can be intrahepatic or extrahepatic:

Intrahepatic obstructions that compress biliary canaliculi include hepatocellular swelling – e.g., hepatic lipidosis, severe corticosteroid hepatopathy in dogs, severe cellular infiltrates (inflammatory or neoplastic) particularly those around the portal area and biliary tree, solid tumours, e.g., primary to the liver (e.g., cholangiocarcinoma) or metastatic, fibrosis around the biliary system, choleliths, parasites (e.g., *Fasciola hepatica*), bile sludging in canaliculi (e.g., severe dehydration in cats).

Extrahepatic obstructions affecting the extrahepatic biliary system can be caused by tumours of the pancreas, biliary tract, or duodenum; inflammation, e.g., pancreatitis; fibrosis, e.g., secondary to chronic recurrent pancreatitis; choleliths; gall bladder mucocele.

2. **"Functional" cholestasis**

Functional cholestasis involves defects in the transporters needed for the active transport of bile acids and bilirubin into the canaliculi. However, recent evidence indicates that even in structural problems, cholestasis results from downregulated transporters. These are ATP-dependent, and excretion is the rate-limiting step in bilirubin metabolism, e.g., Na/K ATPase. Functional cholestasis can occur if drugs, hormones, cytokines, or endotoxins interfere with the hepatocyte transporters, responsible for bilirubin excretion (MRP2). Anorexia in horses and, to a lesser extent, cats cause functional cholestasis.

CHOLESTASIS ENZYMES

ALP Alkaline phosphatase

- ALP is a membrane-bound enzyme that is present in quite a few tissues as different isoforms or isoenzymes, including:
    - liver hepatocytes and biliary epithelium
    - osteoblasts in bone
    - intestinal epithelium
    - renal epithelium
    - mammary gland
    - placenta
- The serum ALP activity measured in the lab is a combination of all the different forms. Isoform differentiation is achieved by electrophoresis or inhibition methods (levamisole or heat). Isoforms or isoenzymes have a minor difference in amino acid sequence but catalyze the same reaction.
- In the dog and cat, the intestinal and renal isoenzyme of ALP constitutes only a small fraction of total serum ALP activity due to the short half-life of only ~6 minutes in serum.
- Similarly, the placental isoenzyme of ALP, which may be increased in late-term pregnancy in animals, also has a plasma clearance half-life of only 6 minutes. Placental ALP increases total

serum ALP in pregnant humans and sometimes in late pregnancy in cats (but not dogs or horses).

- Increased intra-biliary pressure induces an increased ALP production by hepatocytes and bile duct epithelial cells. Cholestasis also causes solubilization of ALP molecules attached to cell membranes and then increased release of these molecules into the blood.
- ALP half-life ~ 3 days dog, < 6 hours cat
- ALP in dogs is highly sensitive and increases often precede hyperbilirubinaemia. Dogs have a corticosteroid-induced isoform (C-ALP), and thus ALP in this species lacks specificity for cholestasis. Bilirubinuria also precedes hyperbilirubinaemia in most dogs.
- ALP in cats has poor diagnostic sensitivity for detecting cholestasis, and they are typically icteric before ALP increases (in cats, GGT is more sensitive). The exception is with hepatic lipidosis in cats, where ALP rises earlier and to a higher degree than GGT. ALP in cats often increases with hyperthyroidism which is primarily due to the bone isoform induction. Given the short half-life in cats and high specificity for cholestasis, any increase in ALP is considered significant.
- ALP in horses has poor diagnostic sensitivity for detecting cholestasis, and they are typically icteric before ALP increases.
- ALP is not commonly used in large animals as ALP has wide reference ranges in cattle, horses, sheep, and goats; it is not a sensitive indicator of biliary disease. GGT is much better in these species.
- Increases in the bone isoenzyme of ALP are due to increased osteoblastic activity. In young, rapidly growing animals, bone ALP is maybe 2-10 times normal. Decrease from 3m, normal 15m. Increases in bone ALP may also be seen with lytic or proliferative bone lesions, fractures, or animals with active bone resorption, so in things like **primary or secondary hyperparathyroidism**. The increases in ALP in these diseases are generally mild, though ALP is not a sensitive indicator of bone disease.
- Genetic causes of ALP increase. Siberian husky and Scottish terriers can have familial increased in ALP
- ALP will be induced by some drugs used for seizure control, e.g., Phenobarbitone, Phenytoin – Dilantin, primidone. The degree of induction depends on dose and duration; however, levels can increase in 12-24 hours and may last for 2-4 weeks after removing the drug. They can also cause hepatocyte injury and thus increases ALT/AST.
- **Corticosteroid**-induced ALP in dogs reflects upregulation of a specific gene in canine hepatocytes to produce this unique enzyme (distinct from isoenzyme upregulated in response to cholestasis). The increased level does not correlate with the severity of steroid hepatopathy (glycogen) and can vary from 2x to 1000x normal. Increases can occur with corticosteroid treatment, e.g., prednisolone, dexamethasone, or endogenous hypercortisolaemia (stress, hyperadrenocorticism). Typically, C-ALP elevations start ~ 7d after initiation of therapy, but L-ALP levels can increase earlier than this as corticosteroids also induce L-ALP. If hypercortisolaemia is the cause of ALP increase, we expect a concurrent stress leukogram. Treatment with corticosteroids leads to a substantial increase in ALP, which may persist for weeks to months after treatment is discontinued.

GGT Gamma-glutamyl transferase

- GGT may be increased to a greater degree than ALP in diseases associated with biliary hyperplasia in the absence of cholestasis (e.g., biliary carcinoma, pyrrolizidine toxicosis, sporodesmin, or *Fasciola* infections in cattle).
- GGT is usually associated with cell membranes where it catalyses the transfer of glutamyl groups between peptides, and it is involved in glutathione reactions. It has the highest concentrations in the pancreas and kidneys. Still, it is also present in biliary epithelial cells

and mammary epithelial cells, particularly during lactation and in the spleen, heart, brain, seminal vesicles, and gallbladder.

- Increased GGT activity in serum is generally the result of enzyme induction involving hepatocytes or biliary epithelial cells. However, with acute hepatic injury, GGT may increase rapidly, possibly due to the release of membrane fragments to which GGT is attached.
- In large animals, GGT is a sensitive test for biliary hyperplasia (it is a good marker for pyrrolizidine alkaloid toxicity in ruminants) and cholestasis (uncommon in large animals, except cholangiohepatitis and cholelithiasis in horses). Overall, GGT is considered a better marker of biliary tract disorders in large animals than ALP.
- GGT concentrations in serum may also be elevated in response to many drugs and toxins. The usual mechanism for this effect is induction of the enzyme leading to increased production and release into the circulation. Medications that lead to increased GGT may also increase ALP, although this does not always occur. e.g., Dilantin, phenobarbitone, trimethoprim/sulpha, erythromycin, and flucloxacillin. Circulating levels may be reduced by cimetidine therapy. GGT levels will show a significant reduction one to two weeks after cessation of a causative agent.
- In dogs, increased serum GGT activity may be seen in dogs treated with glucocorticoids both due to induction and the effects of a steroid hepatopathy.
- Colostrum in all species, except for horses, contains high GGT concentrations. Increases in GGT occur within 24 hours of suckling and are a sensitive indicator of passive transfer. Very high activity of GGT may be observed (up to 1000 x adult levels in 2–3-day old puppies) that gradually decrease with time (over ten days in dogs, 4- 6 weeks in lambs and calves) and is an expected finding in a neonate (i.e., does not indicate hepatobiliary disease)' In calves, the increase in GGT has been used to predict the efficacy of passive transfer however total solids protein appears more accurate and is easier and cheaper to check. IgG measurement is the gold standard test. Note that increases in ALP activity do occur after suckling. This is thought to be derived from intestinal sources in the neonate and is not due to colostrum itself.
- GGT is expressed on the membranes of proximal renal tubular epithelial cells. Cell injury causes GGT to be shed into the urine but not into the blood. The urinary GGT to creatinine ratio has been studied as an early indicator of renal tubular injury, primarily due to aminoglycoside toxicity.

TESTS FOR HEPATIC DYSFUNCTION

- the ability of the liver to clear ammonia; produced daily from amino acid metabolism and must be converted to urea by the liver
- the ability of the liver to clear bile acids which undergo enterohepatic circulation and are efficiently removed from the blood by a normally functioning liver
- the ability of the liver to conjugate bilirubin – requires 80-90% dysfunction to see reduced conjugation
- evaluation of liver synthetic ability; measures substances normally produced by the liver; albumin, cholesterol, urea, glucose, coagulation factors, antithrombin, protein C, however levels of these substances are influenced by many non-hepatic factors, so not liver-specific tests

Hepatic failure occurs when there is a substantial loss of liver tissue (> 70-80%), and thus significant liver disease can be present without evidence of hepatic dysfunction.

Bilirubin

Hyperbilirubinaemia occurs due to one or more of three mechanisms:

1. Pre-hepatic – haemolysis (moderate to severe anaemia before hyperbilirubinaemia will occur)
2. Hepatic (cholestasis)– common, decreased uptake/conjugation/secretion (E dep, rate-limiting) of bilirubin by hepatocytes, anorexia/sepsis
3. Post hepatic (cholestasis) – biliary obstruction

There are three forms of bilirubin in blood:

1. **Unconjugated** (indirect): This is bound to albumin and is the dominant form of total bilirubin in the blood.
2. **Conjugated** (direct): This is water-soluble and is seen in very small amounts in blood because it is normally excreted into bile. It is also the form of bilirubin seen in urine (which is not a normal finding in any species, other than the dog, in which 1+ bilirubinuria may be seen in concentrated urine or a USG > 1.030 [and maybe up to 2+ in highly concentrated urine, > 1.040]).
3. **Delta bilirubin** (or biliprotein), a small fraction in health that is conjugated bilirubin bound to proteins. Delta bilirubin increases in serum when hepatic excretion of conjugated bilirubin is impaired (cholestasis), and the liver retains intact conjugation mechanisms. It has a long half-life and is not excreted in the urine (as it is protein bound). Delta bilirubin may be responsible for a persistent bilirubinaemia without bilirubinuria seen in some animals with cholestasis. It is not routinely individually measured.

Measurement of total bilirubin (primarily direct + indirect) and the bilirubin "split" (direct and indirect bilirubin) in the blood (and detection of bilirubin in urine) can occasionally be helpful in interpreting changes in test results. For instance, increased breakdown of haemoglobin (e.g., severe haemolytic anaemia) will increase the production of mostly unconjugated bilirubin early in the disease. In contrast, an obstruction to bile flow will increase conjugated bilirubin within hepatocytes, which will then be refluxed back into the blood (and spill into the urine), resulting in an increase in total bilirubin, that is usually primarily due to conjugated (or direct) bilirubin. However, in most animals with icterus, by the time the disease is diagnosed the total bilirubin is usually a mix of both conjugated and unconjugated.

Bile acids

- Typically, the liver efficiently clears bile acids from the portal circulation on their first pass through the liver. Hence only a mild post-prandial increase in serum bile acids is seen in healthy animals.
- Increased levels of serum bile acids in the serum can reflect:
    - gall bladder contraction leading to an increased load of bile acids in the intestine and thus increased absorption. This is usually associated with a meal (CCK mediated) but can also occur with excitement in some animals
    - reduced hepatic uptake from the blood due to alterations in enterohepatic circulation (portosystemic shunts)
    - reduced hepatic uptake due to hepatic dysfunction
    - cholestasis –in animals with evidence of cholestasis causing hyperbilirubinaemia then bile acids are NOT helpful to assess hepatic function
- In dogs and cats, the basic procedure for measuring bile acids involves collecting a serum sample after a 12 hour fast, feeding a small amount of food e.g., few spoonful of a high fat

food (such as Hills a/d®) and then collection of another blood sample 2 hours later (the post prandial sample).
- Animals without gallbladders include horses, deer, rats, camelids, some birds, and elephants. In these animals, only a single bile acid sample is taken as there is no advantage in taking a post-prandial sample.

Ammonia

- Ammonia is produced in the GI tract by the breakdown of amino acids and urea (dietary or blood proteins) by gastrointestinal microflora. Some ammonia is also produced during amino acid metabolism in cells (nucleic acids, glutamine, glutamate). The ammonia produced in the gastrointestinal tract is transported by the portal circulation to the liver converted to urea via the hepatic urea cycle. The urea then enters the systemic circulation and is excreted in the urine.
- Increased plasma ammonia concentrations most commonly are found in animals with portosystemic shunts, as the ammonia bypasses the liver and the urea cycle in the shunt, straight into the systemic circulation resulting in hyperammonaemia.
- Increased blood ammonia concentrations can also occur with the loss of 60-70% or more of hepatic functional mass due to decreased liver uptake of ammonia for the portal circulation and decreased ability to convert ammonia to urea.
- In dogs and cats, high ammonia is slightly less sensitive than fasted bile acids but more specific for PSS. Measurement of post-prandial bile acid concentrations may have improved sensitivity at the expense of specificity (particularly in dogs).
- The animal must be fasted for at least 8 hours, after which blood is collected and must immediately be placed on ice, with the plasma separated within 30 minutes. After which, the essay must be completed within 60 minutes, ideally 30 minutes.
- Ammonia is volatile after collection**.** Arterial heparinizes samples are preferred, separated from RBC within 30min and kept on ice if not assayed immediately (3 hr maximum storage) or freeze and transport frozen (on dry ice).
- Because of the technical difficulties involved in these tests, serum bile acid concentrations are preferable.

Protein C

- Protein C can act as a biomarker of hepatic function and hepatoportal perfusion. This is because protein C concentrations appear to be governed by hepatic portal flow and are not only influenced by the synthetic capability of the liver.
- It helps differentiate PSS from microvascular dysplasia (MVD) since portal blood flow is low in PSS and normal in the MVD.
- It can be helpful in monitoring response to ligation of portosystemic vascular anomalies
- Not in common usage (yet)

Urinalysis

Bilirubinuria

- Dogs have a very low renal threshold for bilirubin and their renal tubular cells can convert haeme (resorbed haemoglobin) to bilirubin which they conjugate and excrete in the urine. A trace or 1+ bilirubin on a dipstick from concentrated urine of a dog is considered normal, but in other species would indicate cholestatic liver disease.
- In the intestine, conjugated bilirubin is converted by intestinal bacteria (bacterial proteases) to urobilinogen which is colourless. About 90% of urobilinogen is excreted in the faeces as stercobilin, which gives faeces the brown colour, and about 10% is reabsorbed from the intestine and excreted as urobilin by the kidneys, giving urine its yellow colour. The presence of urobilinogen in the urine indicates that the hepatobiliary system is intact. However, dogs and cats can have negative urobilinogen results when healthy. In a jaundiced animal, negative dipstick results suggest biliary obstruction.
- With marked bilirubinuria, crystals may appear, and bilirubin staining of cells in the urine occurs.
- In all species, bilirubinuria may precede an increase in serum bilirubin in hepatic/cholestatic disorders (conjugated)

Ammonium biurate crystalluria

- Animals with hyperammonaemia may have ammonium biurate crystals in their urine – which are brown/yellow-brown spherical crystals with irregular protrusions "thorny apples."

Haemostatic abnormalities with Liver Disease.

1. **Coagulation factor deficiencies** – due to reduced coagulation factor synthesis (except FVIII). It is detected by prolonged PT and/or APTT coagulation tests (this will be covered in the cardiovascular unit). Deficiencies also occur in antithrombotic factors too.
2. **Vit K dependant coagulopathy** develops with obstructive jaundice. The absorption of fat-soluble vitamins is dependent on the intestinal emulsification of fats by bile acids. Reduced bile flow results in decreased absorption of these fat-soluble vitamins, especially vitamin K. Vitamin K is required for the activation of coagulation factors II, VII, IX, and X and the inhibitors, protein C and protein S. This may result in a prolonged PT (may be affected before APTT, because of the short half-life of FVII) and/or APTT or low activity of FVII: C. May not see clinical bleeding.
3. **Mild thrombocytopenia –** due to reduced thrombopoietin synthesis by the liver
4. **Acquired platelet dysfunction –** poorly understood

Cytology

Abdominal Fluid Analysis: Animals with acquired defects in hepatic portal flow, e.g., those with fibrosis secondary to chronic active hepatitis, can develop a transudative effusion (low or high protein) due to portal hypertension. Animals with congenital PSS do not usually develop ascites.

Liver FNA: can help diagnose neoplastic disease (particularly metastatic disease), inflammatory disease, e.g., hepatic abscess, and vacuolar hepatopathies, e.g., hepatic lipidosis, corticosteroid hepatopathy.