SEVC SOUTHERN EUROPEAN VETERINARY CONFERENCE
SEVILLE, 23-25 October 2025

A DIAGNOSTIC APPROACH TO CANINE HEPATOBILIARY DISEASE

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A SYSTEMATIC APPROACH

A systematic approach is crucial in the investigation of elevated hepatobiliary enzyme activities. This is due to the liver's susceptibility to secondary changes incurred by disease elsewhere in the body (reactive/secondary hepatopathies), as well as the high number of differential diagnoses for primary hepatopathies. It is essential to differentiate primary from reactive hepatopathies early during investigations to ensure prompt diagnosis and treatment. Reactive hepatopathy is the most common hepatobiliary disease, followed by chronic hepatitis, reversible hepatocellular injury (acute hepatitis) and hepatocellular neoplasia[1]. Elevations in hepatobiliary enzyme activities greater than 3-4x the reference interval are suggestive of a primary hepatopathy, and the identification of clinical signs not associated with hepatobiliary disease is suggestive of probable reactive hepatopathy; for example, weight gain, as is seen in hypothyroidism. Common secondary hepatopathies include hyperadrenocorticism, diabetes mellitus, gastrointestinal disease, sepsis and medications such as glucocorticoids and phenobarbital. Management of the cause of reactive hepatopathy will usually lead to resolution of liver enzyme activity elevations. It is also essential to deduce early if a hepatopathy is acute or chronic in duration. Acute hepatitis should be expected in cases with rapid onset of clinical signs in a previously healthy animal, and where hepatomegaly is identified. Chronic hepatopathy is usually associated with an insidious onset of signs and microhepatica, however signs can be subtle enough that an owner's perception is of acute onset. As such, a thorough clinical history is essential in the investigation of hepatobiliary changes, including a detailed dietary history, medication/supplement use, toxin exposure, vaccination, antiparasitic treatment and travel histories. Interrogation for potential unrecognized chronic signs such as weight loss and 'slowing down' should also be performed.

CLINICAL SIGNS

Early clinical signs of hepatobiliary disease are non-specific, including lethargy, hyporexia, vomiting, polyuria/polydipsia and weight loss. With advanced disease, signs such as jaundice, ascites, bleeding diathesis and neurological signs associated with hepatic encephalopathy may become evident.

INITIAL DIAGNOSTICS

Assessment of liver enzymes activities is the first stage of investigations where liver disease is suspected. It is important to recognize that these are not functional liver tests and are not prognostic. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are markers of hepatocellular damage. Alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are markers of intra- and extra-hepatic cholestasis, respectively. ALT has a half-life of 50-60 hours and 80-100% sensitivity for hepatic necrosis and failure but is only 50-80% sensitive for other hepatic diseases. ALP is 86% sensitive for hepatobiliary disease, but only 46% specific, largely because of corticosteroid-induced isoenzyme. Specificity is increased when concurrent GGT elevations are seen (91%). Markers of reduced liver function include low urea, hypoglycemia, hypoalbuminemia, hypercholesterolemia (cholestasis) or hypocholesterolemia (reduced hepatic production) and hyperbilirubinemia. In isolation, these markers are poorly specific, but their specificity increases with the number of abnormal findings.

There are no findings on complete blood count specific to liver disease. Most common findings include anemia (regenerative or non-regenerative; due to gastrointestinal ulceration, haemorrhage or anemia of chronic disease), neutrophilic leukocytosis (due to inflammation, infection or necrosis) and

thrombocytopenia (due to platelet sequestration and/or destruction). Red blood cells are commonly microcytic and hypochromic due to impaired iron transport.

IMAGING

Ultrasonography is an essential tool in investigation of hepatobiliary disease. It allows assessment of the hepatic parenchyma (and size), the biliary tract, hepatic vasculature and extrahepatic organs that may be associated with reactive hepatopathy, such as the pancreas. It also allows targeted collection of samples via fine needle aspiration or cholecystocentesis. However, it is important to remember that ultrasound is neither sensitive nor specific for certain liver conditions. Dog with end-stage cirrhosis or lymphoma can have a normal appearing liver, whilst benign changes (such as nodular hyperplasia) can look like a mass or 'bunch of grapes'. An ultrasonographic diagnosis is not a histological diagnosis, and no dog should be euthanized based on ultrasonographic appearance alone. Computed tomography (CT) is becoming more widely available. It is useful in assessment of hepatic size, in identifying mass lesions and portosystemic shunting, and in surgical planning. Radiography is of limited utility except to provide information of approximate hepatic mass.

ADVANCED DIAGNOSTICS

Specific assessment of liver function is performed via bile acid stimulation testing. Pre- and post-prandial serum bile acids >100μmol/L is up to 100% sensitive and 95-100% specific for portosystemic shunting[2] with cirrhosis also being associated with high resting bile acids (median 98μmol/L) and other liver diseases having relatively lower bile acid elevations[3]. Bile acid stimulation testing can be complicated in the presence of a higher resting sample compared to the post-prandial specimen. This can occur due to interdigestive gall bladder contraction, severe ileal disease (affecting bile acid reabsorption), delayed gastric emptying or an insufficiently stimulatory meal. Resting serum ammonia is another marker of hepatic dysfunction. Over 70% of liver function must be lost before ammonia elevations are seen, making it relatively sensitive for end-stage liver failure or portosystemic shunts. As this test becomes more available to the general practitioner, it is essential to follow recommended sample processing and quality control measures. Ammonia measurements are rapidly affected by exposure to air, the presence of haemolysis and the passage of time. Blood should be collected into lithium heparinized tubes and centrifuged immediately following collection. Minimal exposure to air is essential and the tube should remain capped unless the sample is being handled.

Coagulation testing is recommended in any patient with a bleeding diathesis or prior to liver biopsy, as the liver produces all clotting factors save factor VIII. Loss of liver function is typically associated with prolongation in prothrombin time (PT), but spontaneous haemorrhage is rare.

Fine needle aspiration (FNA) should be considered in diagnosis of liver disease, although clinicians should be aware of the limitations, including low cell yield causing poorly representative samples, a lack of information on tissue architecture, and a relatively low agreement between cytology and histology results (30-61%)[4]. Only 50% of patients with a final diagnosis of hepatic neoplasia on histology had evidence of neoplastic cells on cytology in a 2013 paper[4]. FNAs are most useful for diffuse infiltrative disease such as lymphoma, or where a focal lesion is identified by ultrasound.

Infectious disease testing requires an understanding of the patient's exposure risk, based upon travel history and local disease prevalence. Leptospirosis is a common cause of acute and, less commonly, chronic hepatitis. Combined blood and urine PCR testing and paired serology are recommended to diagnose this disease. Recently available point-of-care antibody ELISA testing has high specificity but is affected by recent vaccination and can be negative in acute disease due to the lag in antibody production. Most other infections affecting the liver either do so as part of a multisystemic disease (e.g., leishmaniasis, mycobacterial disease) or can be diagnosed from bacteriology and PCR of hepatic biopsy tissue.

LIVER BIOPSY

Liver biopsy is an essential part of diagnosis of chronic liver conditions and some neoplasias and can also be considered in acute hepatitis cases of unknown etiology. Before liver biopsy acquisition, it is essential to ensure an adequate platelet count, PT and activated partial thromboplastin time (aPTT). Thrombocytopenia is more commonly associated with major hemorrhagic complications[5]. Elevated PT

should prompt delay in sampling until subcutaneous vitamin K has been provided for a minimum of 36-48 hours or until after a fresh frozen plasma transfusion is administered. Even with appropriate pre-biopsy considerations, there remains a risk of haemorrhage, and for high-risk patients, ideally the patient should have been blood typed with an appropriate unit of pRBCs available. A variety of liver biopsy procedures are available. Open laparotomy allows improved visualization and hemostatic control, allows collection of larger sample sizes and has minimal equipment requirements. However, it is associated with prolonged recovery times, increased post-operative pain and increased costs. Percutaneous ultrasound-guided needle biopsy techniques allow targeting of focal lesions, have minimal equipment requirements and avoid open surgery. They are associated with smaller sample sizes, collection is difficult in the presence of microhepatica and ascites and there is a risk of haemorrhage, which may require conversion to open laparotomy. The current gold standard of liver biopsy acquisition is via laparoscopic techniques. These offer excellent visualization, improved hemostatic control, short recovery time and collection of larger sample sizes. However, they require specialist training and equipment and have an increased cost in most facilities.

Whichever biopsy technique is used, it is recommended to collect 2-5 samples from at least 2 different liver lobes for fixation in 10% formalin. These are used for histopathology and special stains including rhodanine (copper distribution), acid fast stains (mycobacterial disease), periodic acid Schiff stains (fungal disease) and gram staining and fluorescence in situ hybridization (FISH) for bacterial disease. Selection of stains depends on clinical suspicion of disease. A fresh biopsy sample should be collected for aerobic and anaerobic culture, and a fresh frozen sample should be frozen for potential future testing, including copper quantification via atomic absorption spectroscopy and microbial PCRs.

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