Diagnostic testing for liver disease

Careful selection and interpretation of diagnostic tests is key in determining cause and treatment.

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Once the preliminary diagnostic testing suggests that a Pet may have liver disease (See "Indicators of liver disease," page 24), further tests are needed to document whether the liver disease is primary or secondary, to determine the degree of liver dysfunction, and finally to reach a definitive diagnosis. The liver has a large reserve capacity and ability to regenerate. A significant amount of liver function must be lost before the Pet shows overt clinical signs of hepatobiliary disease, such as icterus.[1] Whether the Pet shows clinical signs of liver dysfunction depends upon the extent of the disease and the rate of development (*Figure 1*, page 36). This article discusses the arsenal of diagnostic tests used when trying to determine the cause of liver disease.

Serum bile acids

Bile acids (cholic and chenodeoxycholic acids) are synthesized by hepatocytes from cholesterol. They are secreted into the bile canaliculi after conjugation with taurine or glycine and are stored in the gall bladder. After entering the intestinal lumen subsequent to gallbladder contraction, bile acids are reabsorbed in the ileum and cleared from the portal circulation on the first pass through the liver. Thus, bile acids present in the serum represent the bile acids that were not cleared by the liver (the "spill-over"). Impaired hepatic function results in decreased first pass clearance of bile acids from the portal blood and an increase in the measured serum bile acids concentration. In fasting Pets, portal bile acids concentration is usually low, so serum bile acids may be normal despite impaired hepatic function. Food, especially fat, stimulates gallbladder contraction, causing a bolus of bile acids to enter the intestinal tract and then the portal circulation. Even Pets with normal hepatic function experience a slight elevation of serum bile acids concentrations in the post-prandial state due to the spill-over effect, normally lasting for a few hours. However, in Pets with impaired hepatic function, serum levels of bile acids increase markedly after a meal. The maximal information from bile acids testing is always obtained using a stimulation test (outlined below).[2]

Figure 1

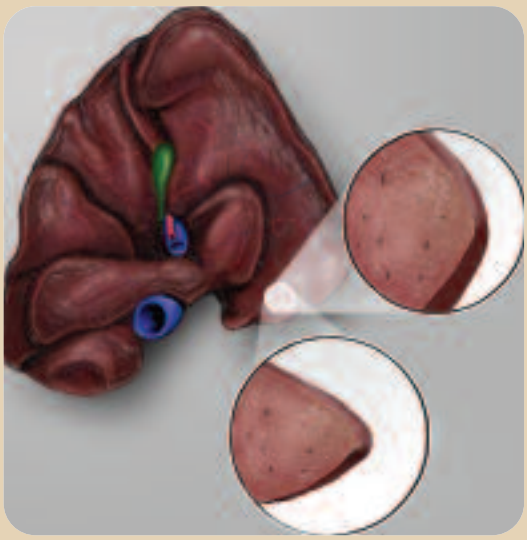

Diagram of liver shows rounded margin (normal/bottom; swollen/top).

Elevated serum bile acids concentration is usually associated with hepatic dysfunction. A bile acids stimulation test should be performed in any Pet with clinical or biochemical findings supporting the differential diagnosis of liver dysfunction. Common causes of elevated serum bile acids concentrations include: portosystemic shunts (PSS), hepatic cirrhosis and hepatocellular disease caused by diffuse inflammation or necrosis.[3,4] Focal hepatic diseases, such as certain forms of hepatic neoplasia, may not impair hepatic clearance of bile acids sufficiently to cause a measurable increase in serum levels. Thus serious, even terminal, diseases involving the liver may be associated with normal serum bile acids concentrations. It is unnecessary to perform a serum bile acids assay with overt evidence of liver disease, such as icterus (typically when the serum bilirubin exceeds 5 mg/dl) because they will be elevated.

Conditions other than hepatic disease can also alter bile acid metabolism sufficiently to increase or decrease the serum bile acids concentrations, and interfere with the accuracy of the bile acids test.[5] These include: pancreatitis, which may obstruct the common bile duct; gastrointestinal motility changes, which alter the delivery of bile acids to the ileum for absorption; and severe inflammatory bowel disease or lymphosarcoma, which impair the absorption of bile acids. Hemolysis and/or lipemia of the blood sample will interfere with the spectrophotometric assay used for measurement of serum bile acids and markedly complicate end-point determination. Although there is some disagreement on this matter, ursodiol (ursodeoxycholic acid, Actigall®–Watson Phamaceuticals, Inc.) therapy should be discontinued for at least four days prior to bile acids testing because the drug may be measured in the assays.[6,7]

A single, random, markedly elevated serum bile acids concentration makes liver dysfunction very likely; however the sensitivity of detecting hepatic disease with only one sample is significantly lower than using the two sample bile acids stimulation test. There is great day-to-day variation in serum bile acids in some Pets, and a random value in the normal range may merely reflect the absence of a significant enterohepatic challenge at that point in time. A normal serum bile acids concentration without a food challenge cannot be relied upon to rule out the presence of liver disease.

Performance of the bile acids stimulation test

The standard protocol recommended by most reference laboratories includes: 1) 12-hour fast; 2) collect pre-prandial serum sample; 3) feed; 4) collect two-hour post-prandial serum sample.

What to feed and how much to feed has been widely debated. It is now accepted that 2 tablespoons for small dogs and cats and 4 tablespoons for the larger dogs, of a canned maintenance diet, seems sufficient for adequate gallbladder stimulation/contraction. If the Pet is anorexic or refuses to eat in the hospital, force-feeding is required.

Recently, the necessity of the pretest 12-hour fast has been questioned. Some clinicians have argued that a random preprandial sample and two-hour post-prandial sampling is sufficient, and the test is positive if either value is elevated. This is based on the fact that a small percentage of cats and dogs have higher fasting than post-prandial bile acids concentrations.[2] This may be due to gallbladder contraction during fasting, intestinal malabsorption associated with disease or motility changes and bacterial overgrowth causing intestinal bile acids metabolism.

Blood should be collected from the jugular vein with the largest bore needle practical to avoid hemolysis of the sample. Gently place the sample in a plain serum tube (not a serum separator). Allow the sample to clot at least 30 minutes, then centrifuge the sample and remove the serum from the clot and place into a separate plain serum tube. Bile acids are then stable at room temperature for several days.

Interpretation of the bile acids stimulation test

The patient with hepatic dysfunction may have a normal or elevated fasting serum bile acids concentration with an elevated post-prandial bile acids concentration. Serum bile acids concentrations of greater than 25 µmol/L in dogs and 20 µmol/L in cats are considered abnormal.[8] The quantitative association between absolute bile acids concentrations and the cause, degree or severity of liver disease has never been proven. Because serum bile acids concentrations can fluctuate markedly hour-to-hour and day-to-day in the same Pet, serial monitoring of bile acids is of no value in evaluating the activity or progression of liver disease. Complete normalization of serum bile acids after therapy would, however, suggest improvement of liver function. Patients with hepatic dysfunction confirmed by elevated serum bile acid concentrations indicate the need for further diagnostics including, but not limited to, imaging and histopathology.

If there is doubt in evaluating the size of the liver, a few milliliters of barium sulfate can be administered by mouth, which will outline the stomach and allow visualization of its cranial border.

Radiography

Radiographs of the abdomen should be a routine part of any diagnostic workup of a Pet with suspected liver disease. The attributes and limitations of this diagnostic procedure, however, must be appreciated. Radiography, as opposed to ultrasonography, is the best way of determining the size and shape of the liver. Hepatomegaly, microhepatica, asymmetry and the presence of masses that change the shape of the liver can be visualized by the use of radiographs. If there is doubt in evaluating the size of the liver, a few milliliters of barium sulfate can be administered by mouth, which will outline the stomach and allow visualization of its cranial border. The area between the cranial border of the stomach

and the diaphragm is occupied by the liver.

Microhepatica is classically seen in Pets with PSS, although sometimes the change is mild and difficult to appreciate.[9] Liver cirrhosis causes microhepatica, and the shape of the liver may be irregular. Alternatively, some normal Pets appear to have a small liver. Hepatomegaly can be seen in a number of conditions, many of which are not primary liver diseases. The latter include hyperadrenocorticism (Cushing's disease), diabetes mellitus and right-sided heart failure. Primary liver diseases that may cause hepatomegaly include hepatic lipidosis, lymphosarcoma and other types of diffuse neoplasia. Radiographs are of little or no use in evaluating liver parenchyma or changes in the architecture of the liver that are not associated with a change in size or border contour.

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Ultrasonography

Ultrasonography is the best diagnostic imaging modality to evaluate the internal structure of the liver. The liver is found in the abdomen immediately caudal to the diaphragm and cranial to the stomach centrally, the spleen on the left and the right kidney on the right. The liver is bounded ventrally by the falciform fat. Normal hepatic parenchyma has a uniform, medium echogenicity, slightly greater than the right kidney cortex and less than that of the spleen. The uniform normal echo pattern of the normal liver is interrupted only by the hepatic and portal veins (*Figure 2,* page 40). These structures can be differentiated by echogenic walls in the portal veins (for an example of portal hypertension in the liver, see *Figure 3,* page 40) and the ability to trace the hepatic veins to the caudal vena cava. Intrahepatic branches of the hepatic arteries are not normally seen within the liver. The gallbladder is found in the liver just to the right of the midline as a normally round-to-oval anechoic structure. The gallbladder size varies depending on when the Pet last ate, increasing with anorexia or fasting. Echogenic sediment (sludge) may be present in the lumen (*Figure 4,* page 40). Intrahepatic bile ducts are not normally visualized. The extrahepatic biliary ducts are more commonly visualized in cats, with overlying bowel gas typically obstructing their view in normal dogs.

Lesions in the parenchyma of the liver are divided into diffuse (or nonfocal) disease and focal (may also be multifocal). Examples of focal liver disease include cysts, hematomas, abscesses, nodular hyperplasia and certain types of neoplasia. Depending upon the cause, hepatic necrosis may appear as multifocal or diffuse. The detection of focal abnormalities of the liver by ultrasound is excellent because the normal hepatic parenchyma provides a uniform background. Ultrasonography is less valuable in recognizing diffuse liver diseases that cause increased, decreased or mixed overall echogenicity. A biopsy is generally necessary to reach a definitive diagnosis in these diseases and often with multifocal lesions.

One of the most important diagnostic and prognostic uses of ultrasonography is the detection of parenchymal changes compatible with neoplasia.[10] Metastatic neoplasia is more common than primary liver tumors, the latter including hepatocellular adenoma and carcinoma and cholangiocellular

Figure 2

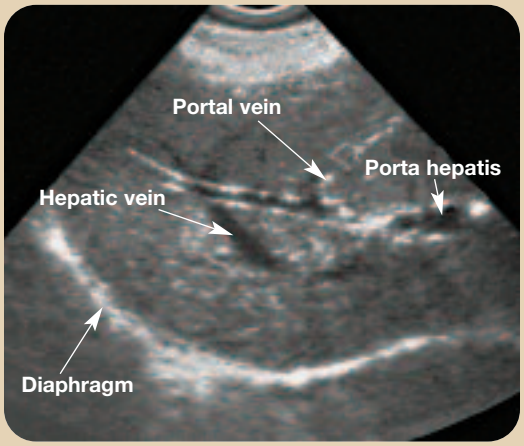

Figure 2. Ultrasound image shows normal liver.

Figure 3

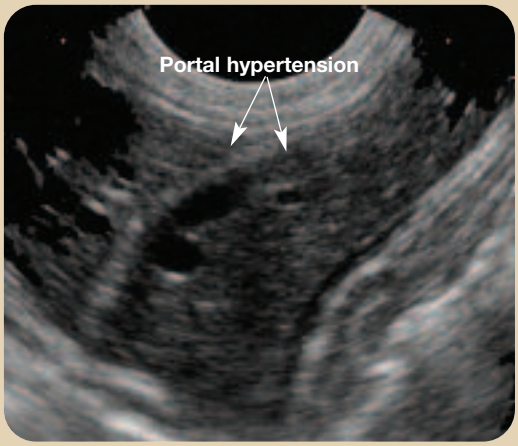

Figure 3. Ultrasonographic appearance of portal hypertension in the liver.

Figure 4

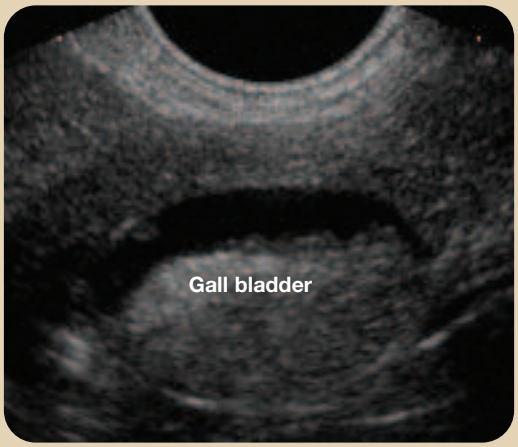

Figure 4. This gallbladder shows the presence of sludge as assessed by ultrasonography.

adenoma and carcinoma. Metastatic tumors are generally carcinomas that may originate from the stomach, intestines, pancreas, mammary gland, spleen (*e.g.*, hemangiosarcoma) or lymphoid tissue (*e.g.*, lymphosarcoma).[11] The type of neoplasia, whether primary or metastatic, cannot be determined by ultrasonography alone. Definitive diagnosis always depends upon identification of the cell type via cytologic examination of needle aspirates or histopathologic examination (*Figure 5*, page 42) of liver biopsies. However, the tumor type may be predicted using general guidelines. Hepatic lymphosarcoma in dogs usually presents as focal or multifocal parenchymal hypoechoic lesions associated with peripheral or abdominal lymphadenopathy. Focal or multifocal hyperechoic masses or those with mixed echogenicity are usually metastatic carcinomas.[11] Multifocal "target lesions" having a hyperechoic center and hypoechoic rim are commonly associated with but not limited to hepatic carcinomas. Cats with hepatic lymphosarcoma often have a general overall increase in size and echogenicity of the liver and lymphadenopathy may be absent. Thin-walled hypoechoic lesions in the liver of a Pet usually represent benign cysts or adenomas. A

solitary liver lesion in a Pet without clinical signs is usually benign; however the abdomen should always be thoroughly searched for free fluid, enlarged lymph nodes or masses in other organs.

Diffuse liver diseases that cause either an increase or decrease in overall echogenicity are much more difficult to appreciate. The echogenicity of the liver is compared to that of the adjoining right kidney cortex and the spleen. Significant error may be introduced in evaluation of liver echogenicity by operator inexperience (adjustments of power output and gain settings are critical) and variability of ultrasound equipment. Diseases which may produce an overall decrease in echogenicity of the liver with more pronounced portal vein walls include infiltrative lymphosarcoma, amyloidosis, acute hepatitis and passive venous congestion of the liver caused by cardiac disease. Overall increase in liver echogenicity has been associated with fatty infiltration, steroid hepatopathy, chronic hepatitis, cirrhosis and lymphosarcoma, especially in cats.[12]

Fat accumulation in the liver most often accompanies diabetes mellitus (dogs and cats) or hepatic lipidosis in cats, causing an increase in liver size. Fat accumulation in cats may be associated with obesity or secondary to dietary restriction. Cirrhosis and chronic hepatitis usually reduce overall size of the liver. This being said, liver size is more accurately assessed by radiographs than with ultrasound. Regenerative nodules, common in the small echogenic liver affected by cirrhosis, appear as distinct less echogenic masses with rounded contour. Focal increases in liver echogenicity may be produced by fibrosis or dystrophic calcification.

A unique pattern of mixed echogenicity pattern occurs in the liver of dogs affected by hepatocutaneous syndrome,[13] a disease of unknown etiology that is characterized by erosions, ulcers and adherent crusts of the mucocutaneous junctions, pressure points, and foot pads. The ultrasound findings in the liver include 0.5 to 1.5 cm diameter hypoechoic regions surrounded by hyperechoic borders producing a honeycomb pattern. As discussed below, liver cytology or a biopsy is required to differentiate and definitively diagnose inflammatory, toxic or neoplastic conditions of the liver parenchyma since the ultrasonographic appearance of each of these conditions can be the same.

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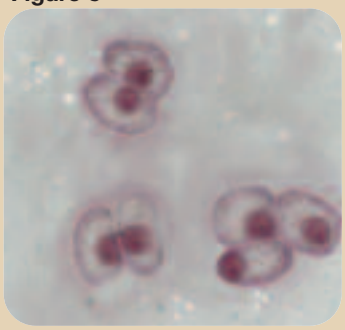
Ultrasonography is useful to evaluate changes in the gallbladder and biliary system associated with extrahepatic biliary obstruction, gallbladder wall disease or biliary calculi. Biliary obstruction outside the liver causes dilation of the gallbladder and common bile duct, wherein the neck becomes more tortuous than when there is only anorexia. If the obstruction persists, extrahepatic bile ducts appear as anechoic tubes with echogenic walls ventral to the gallbladder neck. The bile ducts can be differentiated from portal veins by their sudden changes in lumen size and abrupt branching patterns. The amount of sludge within the gallbladder is variable and not associated with particular disease, and thus should be considered incidental.[14] Biliary tract calculi are rare and likewise often not associated with clinical signs.

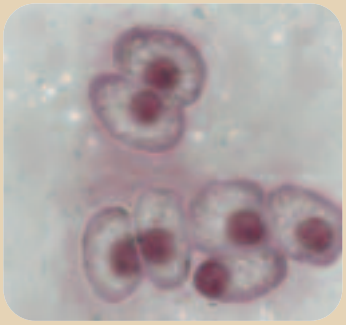
Thickening of the gallbladder wall is a nonspecific finding that may occur in acute

Figure 5



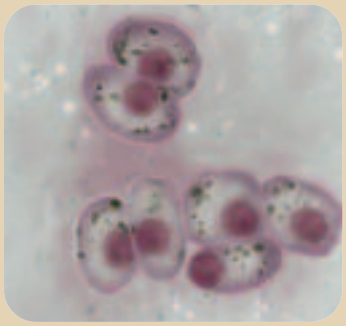
Normal hepatocyte in the liver.

Figure 6



Vacuolar degeneration of hepatocyte cytoplasm in the liver.

Figure 7



Bile accumulation in hepatocytes.

or chronic hepatitis, cholangiohepatitis or cholecystitis. The normally thin echogenic line that represents the gallbladder wall can become thicker or show an echogenic double rim indicating edema within the wall. Gallbladder mucoceles are represented by thickening of the gallbladder wall and presence of sludge or intraluminal masses that do not move with changes in the Pet's position. With gallbladder mucocele or biliary obstruction, gallbladder wall necrosis and perforation can occur if the gallbladder is not surgically removed.

Liver biopsy for histopathology or aspirate for cytology

Ultimately, the best way to reach a definitive diagnosis of liver parenchymal disease is by histopathologic examination of affected tissue. The definitive diagnosis of portosystemic shunts is often only made by use of nuclear scintigraphy. Fine-needle aspiration for cytologic examination is most useful when vacuolar histopathology (*Figure 6*) (*e.g.*, steroid hepatopathy), hepatic lipidosis or diffuse lymphoid neoplasia is suspected. Accumulation of bile within the hepatocytes upon aspiration is suggestive of cholestasis (*Figure 7*). This technique is much less invasive than biopsy and may yield a diagnosis with proper evaluation of other clinical and laboratory data.

Percutaneous needle biopsy is indicated when there is ultrasonographic evidence of diffuse or multifocal hepatic parenchymal disease. Needle biopsy is not performed if there is a chance that the liver condition could be corrected surgically (*e.g.*, single solid or cavitary lesion, PSS), since a better specimen could be obtained at the time of surgery. One study suggests only a 40 percent correlation between 18-gauge needle biopsy and surgical wedge biopsy findings for certain hepatobiliary diseases;[15] therefore, 16- or 14-gauge instruments should be used, if at all possible. In most Pets, heavy sedation and local anesthetic block are sufficient for immobilization for the procedure. General anesthesia is an option but is not required. The biopsy should be ultrasound-

guided to prevent perforation of major blood vessels or the gallbladder, and multiple (at least three) specimens collected. When properly performed, serious complications are rare. Surgically obtained (laparotomy, laparoscopy) biopsy is indicated if the liver is small and/or firm, making it difficult to obtain diagnostic samples by the percutaneous approach.[16]

Coagulation status should be determined prior to liver biopsy since liver disease can lead to clotting factor deficiencies. Although a complete coagulation panel is preferable, activated clotting time and a platelet count are adequate. Platelet function (buccal mucosal bleeding time, or BMBT) should be assessed in breeds prone to von Willebrand disease, or the von Willebrand factor level should be measured. A BMBT provides indirect measurement of platelet function as well as determining if an adequate number of platelets are present. Mild coagulation abnormalities do not preclude liver biopsy, but the latter should be delayed if there is clinical evidence of bleeding or marked coagulation deficiencies. Fresh frozen plasma or vitamin K_1 may be indicated 24 hours before biopsy to normalize coagulation before the procedure. If bleeding is excessive during or after biopsy, fresh whole blood transfusion is indicated.

Peritoneal fluid analysis

Liver diseases that either are associated with portal venous congestion or cause significant hypoalbuminemia (usually less than 2 g/dl) may cause the development of ascites (free fluid in the peritoneal cavity).

Abdominocentesis in affected Pets yields clear, yellow fluid that classically has a total protein less than 3 g/dl (transudate) and low total white cell count (less than 5,000/µl). Ascites is most often seen in association with right-sided congestive heart failure or hepatic cirrhosis, both of which cause acquired portosystemic shunting. Peritoneal fluid exudate (protein concentration greater than 3 gm/dl) should increase the suspicion of neoplasia or infections.

Conclusion

There are many causes of liver disease with widely divergent forms of treatment and overall prognosis. Differentiating the cause in order to guide proper treatment requires careful selection and interpretation of diagnostic tests. The bile acids test is non-invasive and documents liver dysfunction. Imaging of the liver, using radiography and ultrasonography, is invaluable to narrow the list of causes for liver disease. The ultimate diagnosis is usually based on results of liver biopsy. However, even extensive diagnostic testing may not reveal the cause of hepatic disease. Without a definitive diagnosis, empirical therapy could be worthwhile and will be discussed in the next article.

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