Liver Enzyme Elevations in Dogs: Diagnostic Approach*

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Abstract: *Increased liver enzyme activities are sensitive indicators of hepatic disease, but their limited specificity can make their interpretation a challenge. A stepwise approach to diagnosing the source of increased liver enzyme activity involves characterization of the clinical picture, identification of the predominant enzyme activity pattern, elimination of common extrahepatic diseases, and a systematic diagnostic workup for hepatobiliary disease. Comprehensive hepatobiliary testing includes a complete blood count, biochemical profile, and urinalysis; coagulation and liver function tests; abdominal imaging; bile cytology and culture; and liver biopsy for histopathology, culture, and metal analysis.*

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Alterations in circulating liver enzyme activities may present a diagnostic challenge because they are common, often nonspecific biochemical findings. A systematic approach to interpretation of increased liver enzyme activities is outlined in **FIGURE 1**. The first step is to develop an integrated clinical picture based on the patient's signalment, history, clinical signs, and concurrent laboratory abnormalities. Careful evaluation of this picture aids in ranking diagnostic differentials.

Increases in liver enzyme activities are categorized as cholestatic, hepatocellular leakage, or mixed. A cholestatic or inducible pattern is characterized by predominant increases in alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (GGT) activities. A hepatocellular leakage pattern has predominant increases in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) secondary to hepatocyte injury or necrosis. A mixed pattern of liver enzyme activity increase suggests concurrent hepatocellular injury and cholestasis and may be caused by concurrent disease processes or progressive disorders.

The magnitude and duration of increase in liver enzyme activities should be considered in addition to the pattern. Mildly increased activity of a single liver enzyme in a clinically asymptomatic dog may be spurious and should be rechecked. Lipemia and hemolysis may falsely increase activities of serum liver enzymes and should be considered when interpreting laboratory data. Because increases in hepatocellular leakage enzyme activity are proportional to the severity of damage, increases greater than twice the reference interval warrant further scrutiny. Increased activity of multiple liver enzymes, particularly if a hepatocellular or mixed pattern of injury is present, also warrants close attention because of an increased likelihood of severe disease.

Signalment, History, and Physical Examination

Once spurious or self-limiting increases of liver enzyme activities have been ruled

*A companion article, "Liver Enzyme Elevations in Dogs: Physiology and Pathophysiology," begins on page 408.

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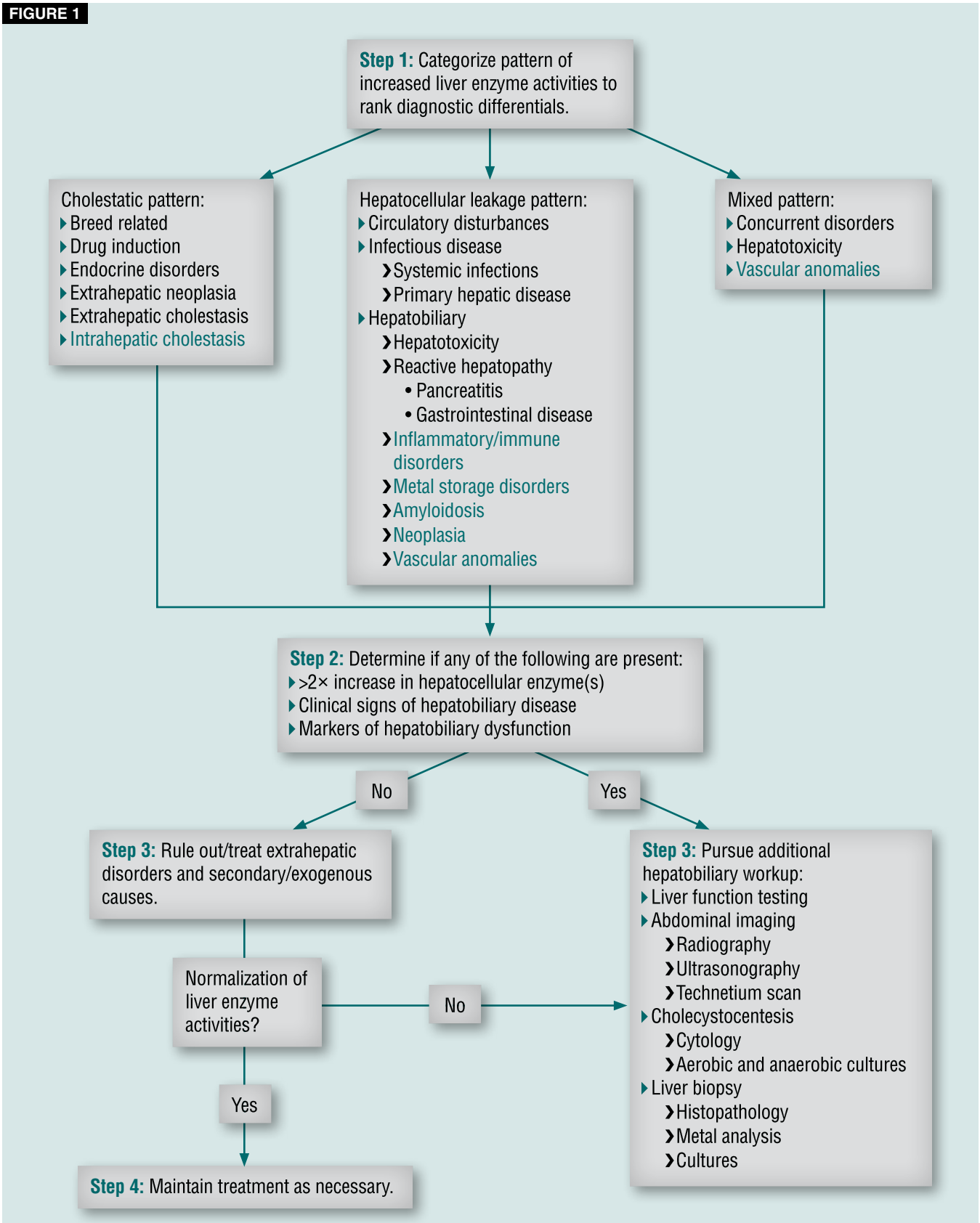
FIGURE 1



Flowchart demonstrating a stepwise approach to the evaluation of increased activities of liver enzymes. Blue differentials are etiologies that may require a comprehensive hepatobiliary evaluation for diagnosis.

BOX 1

Breeds Associated With Increased Activities of Liver Enzymes[a]

Hepatocellular leakage pattern
- Idiopathic hepatitis
  - American and English cocker spaniels
  - Labrador retriever
  - Standard poodle
- Copper-associated hepatitis
  - Bedlington terrier
  - Dalmatian
  - Doberman pinscher
  - Labrador retriever
  - Skye terrier
  - West Highland white terrier
- Amyloidosis
  - Chinese shar-pei

Cholestatic pattern
- Benign hyperphosphatasemia
  - Scottish terrier
  - Siberian husky
- Hyperlipidemia
  - Miniature schnauzer
- Gallbladder disease
  - Shetland sheepdog

[a]This list does not include breeds associated with congenital vascular abnormalities, which commonly have mixed liver enzyme activity patterns.

QuickNotes

Supplements, nutraceuticals, and topical medications are easily overlooked as potential hepatotoxins.

out, the patient should be evaluated for breed associations (**BOX 1**). A careful dietary history may identify risk factors for infection (e.g., raw or undercooked meat) or intoxication (e.g., poor food storage practices, recalled diets). Depending on the geographic location, access to *Amanita* mushrooms and cycad palm plants should be determined. The owner should be carefully questioned about medications to which the patient may have access, including supplements, nutraceuticals, and topical glucocorticoids. Owners may not notice a pet's consumption of prescription medications or know that common items for human use, such as those containing xylitol, can be hepatotoxic.

Diagnosis of drug-related hepatotoxicity can be challenging. The time between initial administration of a medication and detection of hepatic injury can vary greatly, which makes it difficult to establish exposure preceding the onset of injury.[1] Liver injury may worsen for days or weeks following withdrawal of the medication before improvement is seen. Repeat challenge with a suspected hepatotoxin may greatly exacerbate hepatotoxicosis. Alternatively, a patient may develop tolerance for a hepatotoxin and have negative challenge results. If potential hepatotoxins are identified, they should be discontinued and liver enzyme activities reassessed in 2 to 3 weeks.

The patient's vaccination history and potential exposure to infectious agents should be determined. Hepatopathies have been documented with *Leptospira interrogans* serovars Icterohaemorrhagiae and Pomona and *Leptospira kirschneri* serovar Grippotyphosa[2]; additional associations may be identified with increased molecular testing. Canine adenovirus 1 infection, heartworm disease, ehrlichiosis, leishmaniasis, neosporosis, toxoplasmosis, and systemic mycoses should be considered as potential causes of hepatocellular disease.[3,4] In dogs, bacterial infections arise primarily from hematogenous routes and breaks in host defenses, such as periodontal disease.[5]

Lethargy, inappetence, vomiting, and diarrhea are common clinical signs. Icterus, ascites, acholic feces, or hepatic encephalopathy may occur with acute liver failure or progression of chronic disease. Physical examination may be unremarkable or may reveal abnormalities consistent with an extrahepatic disorder. Poor body condition, icterus, abdominal pain, hepatomegaly or microhepatia, and abdominal effusion may suggest hepatic dysfunction. The presence of clinical signs, physical examination abnormalities, or evidence of decreased liver function should prompt an expeditious workup to maximize chances of obtaining a diagnosis and successful therapeutic intervention.

Laboratory Screening

A minimum database of a complete blood count (CBC), biochemical profile, and urinalysis should be obtained for dogs with verified increased activities of liver enzymes.

Hepatobiliary disease can be associated with a variety of CBC abnormalities. Although gastrointestinal bleeding or coagulopathy secondary to hepatobiliary dysfunction may stimulate a regenerative anemia, the chronicity of many hepatic diseases often leads to anemia

of chronic disease, which is nonregenerative. Microcytosis is common with acquired or congenital portosystemic shunts.[6] Thrombocytosis occurs with hyperadrenocorticism,[7] chronic blood loss, and inflammatory disorders,[8] and thrombocytopenia suggests ongoing vasculitis or consumptive processes.

Biochemical changes may aid in the differentiation of extrahepatic and hepatobiliary disease. Concentrations of bilirubin, cholesterol, blood urea nitrogen (BUN), albumin, and glucose provide information about liver function despite low sensitivity and specificity. In the absence of hemolysis or sepsis, hyperbilirubinemia indicates cholestasis due to hepatobiliary disease or posthepatic obstruction. Cholesterol concentrations increase with cholestatic disease and decrease with end-stage liver disease.[9] BUN concentration may be increased secondary to gastrointestinal bleeding or decreased by shunting of portosystemic blood or decreased hepatic production.[9] Creatinine concentration typically remains within the reference interval. With the loss of approximately 70% of functional hepatic parenchyma, hypoalbuminemia may occur.[10] Other causes of hypoalbuminemia should be ruled out, including gastrointestinal disease, protein-losing nephropathy, hemorrhage, vasculitis, inflammation, and prolonged anorexia. Hypoglycemia may occur after loss of 75% of hepatic parenchymal function and is a poor prognostic indicator.[9,11] Other causes of hypoglycemia should be excluded, including congenital portosystemic vascular anomalies, insulinoma, and sepsis.[12]

A variety of abnormalities may be identified on urinalysis. Hyposthenuria and isosthenuria occur frequently with extrahepatic and hepatobiliary diseases. Bilirubinuria with or without hyperbilirubinemia may be identified. A small amount of bilirubinuria can be normal, particularly in male dogs.[13] The presence of urobilinogen can be normal or associated with hepatic disease, while absence may suggest biliary obstruction. Due to urobilinogen's instability and variable excretion, this test has questionable clinical usefulness.[14] Portosystemic shunting and end-stage liver disease may result in ammonium biurate crystal formation. Glucosuria and aminoaciduria, consistent with Fanconi syndrome, have been noted in dogs with copper-storage hepatopathy.[15]

Additional Testing

Thorough evaluation of liver enzyme activity increases may require additional testing for extrahepatic and hepatobiliary diseases after integration of the minimum database with the clinical presentation. Hyperadrenocorticism and hypothyroidism should be considered in dogs with appropriate clinical findings and cholestatic enzyme activity increases. Thyroid hormone testing should be interpreted carefully because sick euthyroid syndrome is common in dogs with chronic disease. Dogs with gastrointestinal signs should be evaluated for pancreatitis and primary gastrointestinal disease. Depending on risk assessment and vaccination history, infectious disease screening may be indicated.

Liver function can be evaluated in a variety of ways. Increased bile acids are extremely sensitive for detecting decreased functional hepatic mass or portosystemic shunting in nonicteric patients. Administration of ceruletide, a cholecystokinin analogue, instead of food may improve the reliability of results in anorectic patients, facilitate standardization of serum bile acid evaluation, and be more sensitive for mild hepatic dysfunction.[16] Urine nonsulfated bile acids have a specificity similar to that of serum bile acids but are less sensitive.[17] Increased ammonia concentration occurs with portosystemic shunting, hepatic failure, and urea cycle enzyme deficiencies and is a useful biomarker for hepatic encephalopathy.[18] Protein C is a circulating anticoagulant protein that is synthesized by the liver. Decreased protein C activity may be a useful biomarker for decreased hepatic function or hepatoportal perfusion.[19] Protein C activity may also be useful for therapeutic monitoring and differentiation between microvascular dysplasia and congenital portosystemic shunts.[19] The liver synthesizes most clotting factors and is responsible for activation of vitamin K–dependent clotting factors. In dogs with chronic hepatitis, prolonged prothrombin time (PT) and partial thromboplastin time (PTT) have been associated with decreased survival times.[20] Buccal mucosal bleeding time is used to assess risk of abnormal platelet function. Because of the fragile balance between procoagulant and anticoagulant factors,[21] we recommend that PT, PTT, and buccal mucosal bleeding time tests be conducted no more than 24 hours before liver biopsy.

*Quick***Notes**

Coagulation testing should be conducted no more than 24 hours before liver biopsy.

Diagnostic Imaging

Survey radiography allows assessment of the size, shape, position, opacity, and margins of the liver. Mineralization of hepatic parenchyma or choleliths may be detected on survey films. Abdominal ultrasonography allows visualization of focal, multifocal, or diffuse lesions within the hepatic parenchyma, although it cannot distinguish pathologic from benign lesions.[22] Ultrasonography also allows assessment of the biliary tract and provides visualization for percutaneous cholecystocentesis, a minimally invasive method of bile collection.[23,24] Gallbladder rupture and iatrogenic inoculation of bile with surface microbes are rare complications,[23] but the procedure is generally regarded as safe. Normal findings on abdominal imaging do not rule out primary hepatobiliary disease.

*Quick***Notes**

When a liver biopsy is performed, samples should be obtained for histopathology, aerobic and anaerobic cultures, and copper and iron quantitation.

Liver Sampling Techniques

The optimal time for liver biopsies is early in the disease course so that they yield useful information rather than demonstrate nonspecific end-stage changes. When selecting a biopsy technique, clinicians should consider the ability to obtain adequate samples for histopathology, aerobic and anaerobic cultures, and copper and iron quantitation. Contraindications to biopsy include coagulopathy and liver failure.

Ultrasound-guided fine-needle aspiration of the liver is easy to perform and minimally invasive. Unfortunately, cytology has been demonstrated to have poor agreement (29% to 66%) with primary histopathologic diagnoses,[25,26] including the diagnosis of neoplasia. In data from two recent studies, 3.6% (2 of 56)[26] and 3.9% (2 of 51)[27] of cases were inappropriately diagnosed with neoplasia on cytology; 14% (2 of 14)[26] and 50% (5 of 10)[27] of cases with histologically confirmed neoplasia were identified cytologically. The poor agreement between liver cytology and histopathology and the potential ramifications of misdiagnoses should be kept in mind when considering fine-needle aspiration findings.

The least invasive method of liver biopsy is percutaneous ultrasound-guided needle biopsy. One disadvantage of this technique is the inability to directly monitor the liver for hemostasis. Needle biopsy findings concurred with findings from wedge biopsies only 48% of the time in one veterinary study.[28] Accuracy may be improved by use of the largest appropriate biopsy instrument and collection of multiple samples. Keyhole surgical biopsies allow collection of large samples and good access for hemostatic control, but the surgeon's ability to evaluate all aspects of the liver, gallbladder, and other organs is limited. Laparoscopy offers excellent visualization and sampling of the liver, gallbladder, and other abdominal organs; provides better hemostatic access than percutaneous sampling; and is less invasive than laparotomy. Widespread use of laparoscopy is limited by requirements for specialized training and equipment. Laparotomy is recommended when surgical cure may be possible and in cases for which full exploration of the abdominal cavity is desired.

Diagnostic Sampling

Histopathologic evaluation of liver biopsy samples remains the gold standard for the diagnosis of hepatobiliary disease, but it can fail to provide a definitive diagnosis. Clinicians often must incorporate information regarding the extent and type (inflammatory, neoplastic, vascular, vacuolar) of pathology into the clinical picture to make a diagnosis.

Quantification of iron and copper concentrations may aid in diagnosis, prognostication, and therapeutic tailoring.[29,30] Abnormally high hepatic copper levels cause oxidative stress and are associated with low hepatic glutathione levels, which contribute to hepatocellular damage.[29] Both inborn errors of metabolism leading to decreased copper excretion and impaired copper excretion due to chronic hepatitis and cholestatic disease may occur in dogs.[29] Abnormal accumulation of iron may be due to increased intestinal absorption or abnormalities of hemoglobin metabolism and delivery of iron to the liver.[31] Accumulation of iron in Kupffer cells has been correlated with inflammation and increased copper levels.[30]

Aerobic and anaerobic cultures of hepatic tissue and bile complete a comprehensive hepatobiliary workup. In findings from one study,[32] biliary cultures were more commonly positive (30%) than hepatic cultures (7%) in dogs. It is unclear whether the biliary system is more susceptible to infection or whether it

is a more sensitive sampling site. Cytologic evaluation of bile may aid in identification of infectious organisms.

Conclusion

Successful interpretation of liver enzyme abnormalities requires integrated evaluation of the patient's clinical picture and diagnostic testing. A thorough diagnostic workup for hepatobiliary disease includes a CBC, biochemical profile, and urinalysis; liver function tests, including coagulation parameters; abdominal radiography and ultrasonography; bile cytology and culture; and hepatic biopsy for histopathology, culture, and metal analysis. Employing a systematic approach facilitates diagnosis of hepatobiliary disease in a timely fashion to improve patient outcome. C

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1. **The _____ of liver enzyme activity increase is not a consideration when evaluating a dog with increased liver enzyme activities.**
   a. magnitude **c.** pattern
   b. duration **d.** sequence

2. **Which of the following may be associated with hepatotoxicosis?**
   a. xylitol **c.** metaldehyde
   b. ethylene glycol **d.** cannabis

3. **Which of the following regarding hepatotoxins is incorrect?**
   a. Pet owners may not consider human foods as potential hepatotoxins.
   b. Nutritional and herbal supplements are often overlooked during the history.
   c. Plants and fungi do not contain hepatotoxins.
   d. Timing between ingestion of a hepatotoxin and liver injury can be highly variable.

4. **_____ has/have not been associated with hepatic disease.**
   a. Canine adenovirus 1
   b. Canine parvovirus
   c. *Leishmania infantum*
   d. *Leptospira interrogans*

5. **_____ is a poor prognostic indicator in patients with hepatic disease.**
   a. Hyperbilirubinemia
   b. Decreased BUN concentration
   c. Hypercholesterolemia
   d. Hypoglycemia

6. **_____ can be used as a biomarker of hepatic encephalopathy.**
   a. Serum bile acids
   b. Urine nonsulfated bile acids
   c. Serum ammonia concentration
   d. Protein C activity

7. **Which statement regarding abdominal imaging is correct?**
   a. A lack of ultrasonographic abnormalities rules out clinically significant hepatic disease.
   b. The ultrasonographic appearance of the liver can be used to distinguish benign from pathologic changes.
   c. Abdominal ultrasonography provides a reliable and noninvasive means to assess the gallbladder and biliary tree.
   d. Seeding of the gallbladder with skin bacteria is a frequent complication of ultrasound-guided cholecystocentesis.

8. **Which is not a contraindication to liver biopsy?**
   a. increased bile acids
   b. poor anesthetic risk
   c. coagulopathy
   d. liver failure

9. **Which is not an advantage of fine-needle aspiration of the liver?**
   a. It allows reliable diagnosis of neoplasia.
   b. It is minimally invasive.
   c. The risk of hemorrhage is limited.
   d. It does not require anesthesia.

10. **When performing a biopsy, samples of liver parenchyma should be collected for all of the following except**
    a. histopathology.
    b. protein C activity.
    c. metal analysis.
    d. bacterial culture.