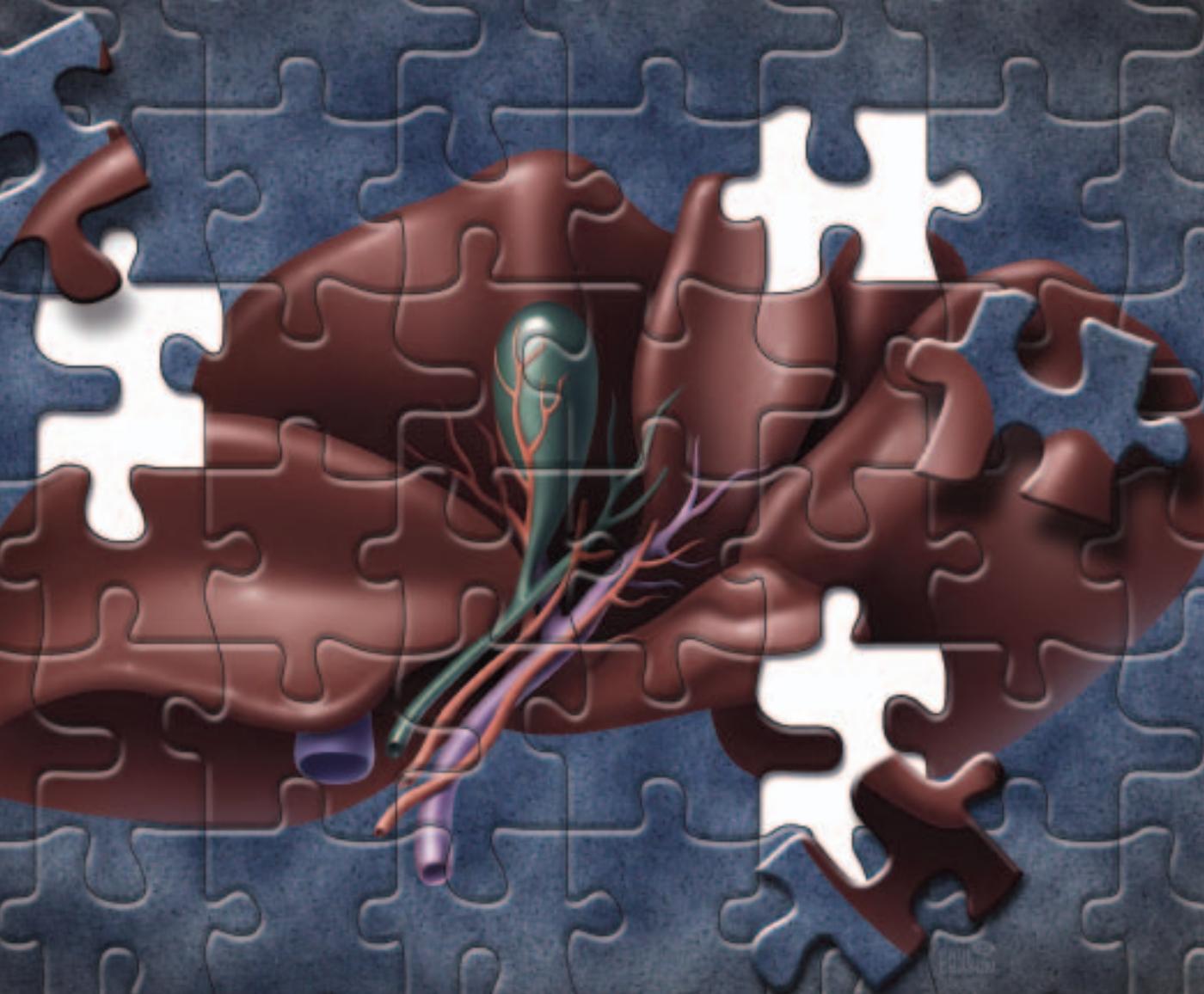


Diagnosing Liver Disease

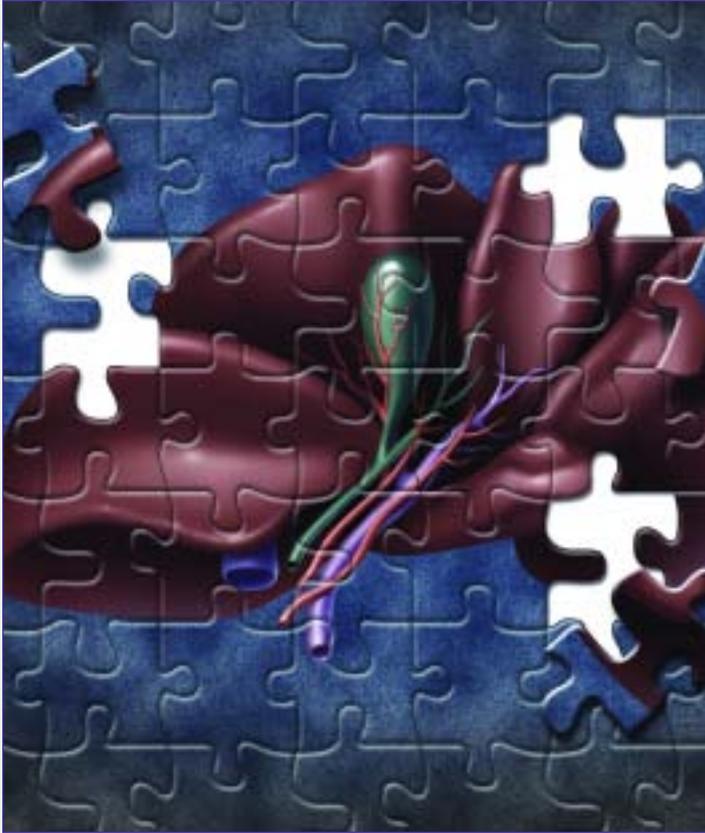
A roundtable discussion



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DIAGNOSING LIVER DISEASE

A ROUNDTABLE DISCUSSION



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Dr. Dennis DeNicola: As every practitioner knows, liver disease can be frustrating to diagnose. Today's roundtable includes internationally recognized experts who share a common goal: to provide practical information that will help readers diagnose hepatic disease in dogs and cats. Let's begin by addressing the various types of liver disease.

Types of liver disease

Dr. David Williams: First, we must differentiate between dogs and cats because liver disease is so different in each species. Also, I categorize liver disease as juvenile-onset and hereditary (different types but with some overlap) vs. adult-onset and acquired. And then acute vs. chronic.

Dr. David Twedt: I classify liver disease by basic histologic findings, such as inflammatory or noninflammatory changes. For example, cholangitis is more common in cats and chronic hepatitis is more com-

mon in dogs. In some dog breeds, abnormal copper accumulation is one cause of hepatitis. I also see a range of acute toxic liver diseases associated with various drugs or other compounds, as well as neoplastic liver disease.

Next, I classify conditions that may be associated with extrahepatic biliary obstructive disease, such as those caused by cholelithiasis or pancreatitis, followed by the vascular diseases, including the congenital portosystemic shunts usually identified in young dogs.

Finally, another common classification is various histologic changes that occur secondary to other systemic and metabolic diseases. Some refer to these liver changes—where the liver is not the primary problem—as reactive hepatopathies. Every day, practitioners must try to determine if the patient has secondary changes or primary liver disease. For example, does the dog have Cushing's syndrome? Does the cat have hyperthyroidism and secondary changes? Or do

they both have primary liver disease? I think the secondary changes are the most common cause of abnormal liver enzyme activities.

Dr. Robert Hawthorne: Practitioners look at an animal's history and physical examination findings and work toward a histologic diagnosis. Many liver cases are presented to us as icteric dogs or cats. But I think we're getting better at catching these liver diseases earlier with biochemical testing. So I classify liver diseases similarly—by species, age, and whether a patient's history reveals a toxic insult or injury. Then I proceed with diagnostic testing to differentiate problems such as vacuolar hepatopathy, cholangiohepatitis, and extrahepatic diseases.

Dr. Sharon Center: I would add another category: cats with hepatic lipidosis and dogs with vacuolar hepatopathies. We see a large number of dogs with a vacuolar

hepatopathy that represents a unique histologic response associated with steroid hormone abnormalities or response to a systemic disease.

Prevalence

DeNicola: What is the prevalence of liver disease in dogs and cats?

Center: Practitioners see a lot of animals with increased liver enzyme activities. These animals may or may not have liver disease because increased enzyme activity may merely reflect the sentinel organ status of the liver and its secondary response to systemic and metabolic conditions. So doctors must perform thorough diagnostic testing to determine which animals truly have liver disease.

Williams: So the question becomes, “What percentage of animals with abnormal liver enzyme activities truly have liver disease?” I suspect it’s less than 10%.

Hawthorne: I agree; the prevalence of primary liver disease is 10% or less.

Causes of liver disease

DeNicola: So let’s first address the most common causes of primary liver disease, starting with infection-based hepatitis. How often do you see that?

Center: In our referral institution, we culture everything, and we find positive cultures (multiple colonies) on our liver biopsies up to 30% of the time. Experimental data^{1,2} indicate that transmural passage of bacteria from the alimentary canal can be an important factor invoking liver injury.

While we do see dogs with hepatic histopathologic lesions similar to lesions classified as viral hepatitis in people, we haven’t identified causal viral infections in veterinary medicine (other than adenovirus, for which we vaccinate).³

DeNicola: Obviously, there are many ways of classifying liver disease. *Table 1* on page 4 includes some of the more common disease entities using a histologic classification scheme. As discussed, the overall impression about the incidence of primary liver disease in animals with suggestive liver enzyme abnormalities is 10% or less.

Clinical signs

DeNicola: Besides icterus, what are other common signs of liver disease?

Hawthorne: Certainly we see the “ain’t doing right” animals with lethargy. We may see elevated enzyme activities, weight loss, or possibly ascites.

Twedt: The signs in dogs with liver failure are quite straightforward: gastrointestinal signs, such as vomiting and diarrhea, and, as the disease progresses,



“Practitioners see a lot of animals with increased enzyme activities. To determine which animals truly have liver disease, doctors must perform thorough diagnostic testing.”

—Sharon Center, DVM, DACVIM

ascites, icterus, and possibly neurologic signs attributed to hepatic encephalopathy. But earlier in the disease process, the signs can be quite vague. I think a lot of dogs have liver disease with no clinical signs. If a blood chemistry profile identifies abnormal liver enzyme activities, then veterinarians can identify liver disease with a proper workup. The liver has a great reserve capacity. So if you see patients with clinical evidence of liver disease, such as ascites or icterus, they likely have advanced disease.

Dr. Jörg Steiner: I categorize clinical signs into groups—subclinical, mild, moderate, and severe.

DeNicola: It is essential, then, that patients with possible liver disease

receive complete physical examinations and diagnostic workups. Something as simple as dental disease may be causing the increased liver enzyme activities. And aside from end-stage liver patients with obvious clinical signs, it’s important to look for cyclic problems and general signs that are not specific to the liver but could be associated with the gastrointestinal tract, kidneys, or the pancreas.

Major enzyme categories

DeNicola: In veterinary schools, liver enzymes are usually categorized as indicators of 1) cholestatic disease and 2) hepatocellular injury or leakage. Is this a reasonable approach?

Center: We should use the phrase “cholestatic-induction enzymes” when referring to alkaline phosphatase (ALKP) based on a collection of investigative studies. The activity of ALKP reflects an induction phenomenon associated with enhanced protein transcription. This phenomenon has been widely studied as a response to corticosteroids (endogenous and exogenous).

DeNicola: I agree. It would help practitioners recognize that 1) induction of the classically identified cholestatic enzymes, such as ALKP and gamma glutamyl transferase (GGT), is not just due to cholestasis and 2) other enzymes, including alanine aminotransferase (ALT), can be induced to a much lesser degree with the same stimuli. Are there additional classification suggestions beyond cholestatic-induction enzymes?

Steiner: Why classify them at all? There are only three or four enzymes, so I just think about how they can be elevated.

Center: For practitioners, it makes a difference because seeing an animal with increased transaminases one week and then observing increased cholestatic enzyme activity a week later suggests that the patient had a hepatic insult during the first week. The enzyme activities collectively can tell a story about the associated pathologic process.



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Table 1

Types of Hepatic Disease

Hepatocellular injury

Reversible injury
Irreversible injury (necrosis)

Hepatitis

Infectious
Noninfectious
Toxic

Obstructive

Intrahepatic
Extrahepatic

Hepatopathy

Vacuolar (dog and cat)
Copper storage (dog)
Hepatic lipidosis (cat)

Vasculopathy

Portosystemic shunt
Microvascular anomaly

Neoplasia

Primary hepatic
Secondary (metastatic)

Hepatic insufficiency

Hepatic cirrhosis

Alanine aminotransferase

DeNicola: Now let's discuss each enzyme and address elevation, liver specificity, and half-life. I'd like to start with ALT. What does an elevation of ALT indicate?

Steiner: Hepatocellular damage. Increased ALT activity is primarily an indication of hepatocellular injury—at least in dogs and cats.

DeNicola: So what about mild increases in ALT? What is clinically relevant?

Twedt: I sometimes see dogs with slight ALT increases and, histologically, they're completely normal. This is often frustrating. Perhaps a specific lesion was missed, or the histology did not reflect changes in hepatocyte membrane permeability with ALT leakage. I think animals with ALT increases and no clinical signs should be followed serially. If the enzymes are two or three times the normal range or persistent and unexplainable, those are the patients I want to investigate further.

DeNicola: What does a marked increase in ALT mean vs. a minor increase?

Twedt: ALT is a sensitive indicator of liver disease but not necessarily specific for a primary disease that we have to treat. We have to keep that in mind. In addition to assessing the enzyme values, we need to evaluate the entire patient for systemic disease and always determine the animal's drug history. Various medications can cause liver changes and increased enzyme activity. We must always perform a physical examination to identify whether the animal has other obvious problems, such as neoplasia. The enzyme values alone don't specify the liver as the problem.

Steiner: I use a twofold and fivefold rule. With a twofold to fivefold ALT elevation in an otherwise normal animal presented for an elective procedure, I watch that patient and recheck it. I may postpone the procedure, but if it's medically indicated—for example, a dental cleaning in a patient that eats poorly because of its dental disease—I do not postpone. With a persistent twofold elevation that is reproducible over two or three months, I get more aggressive diagnostically. If the ALT is initially

higher than a fivefold elevation, I pursue additional diagnostics immediately.

Center: When we find threefold increases in serum ALT activity, we go back with a fresh mindset and review the patient's history, asking specific questions about drugs, herbal or alternative therapies, food changes, and topical treatments. We're particularly alert to changes that can invoke a vacuolar hepatopathy in dogs. This is a major cause of increased liver enzyme activity secondary to a variety of nonhepatic primary health problems.

Increased ALT activity is also common in hyperthyroid cats and cats with subclinical hepatic lipidosis, low-grade cholangiohepatitis, or inflammatory bowel disease.

If ALT activity is increased threefold and the history and physical examination findings suggest a problem, we recommend a liver function test. We do this in our hospital by evaluating paired serum bile acid concentrations (a fasting or random sample followed by a two-hour postprandial sample).

If function tests are abnormal, we recommend abdominal ultrasonography and discuss the possibility of a liver biopsy. The ultrasonogram helps establish liver size; allows us to collect a preliminary hepatic aspiration sample for cytologic inspection; and helps us evaluate the biliary tree, pancreas, and alimentary canal. The ultrasound findings may increase the index of suspicion for certain disorders (e.g., a small liver with irregular margins in chronic hepatitis [dogs]).

If liver function tests are normal, we recheck liver enzymes in two to three weeks. If liver function remains normal but liver enzymes remain abnormal, we recommend an abdominal ultrasound. Ultimately, with chronicity, we may recommend a liver biopsy.

Williams: So the more abnormal the enzyme activity is, the more certain you are that something's going on.

Hawthorne: Many practitioners still regard measurement of ALT and ALKP activities and total bilirubin concentration as liver function tests. They're not.

DeNicola: Right. Practitioners need to do more than a general blood chemistry profile to accurately assess liver function.



Steiner: Out of 100 dogs with a two-fold or threefold ALT elevation, only a few develop liver disease requiring treatment. I'm not saying we shouldn't test for these abnormalities, but I think we need to be cautious about being overaggressive with follow-up diagnostics.

Hawthorne: That's a good point. After practicing for more than 20 years, I find myself getting caught in a trap. My new-graduate colleagues focus on these enzyme elevations and say, "We've got to check them." But the animal is clinically normal. The client then wants to see the older doctor because the older doctor won't run all the tests again. On the other hand, if we don't recheck the enzyme activities six months later, we may have an animal in crisis.

Center: And the records prove that you didn't pursue it.

DeNicola: Let's say a practitioner has a young animal that needs a dental cleaning or lipoma removal. The physical examination findings are normal, but presurgical blood tests reveal a twofold to threefold elevation in ALT activity. What should happen next?

Center: Too often the veterinarian ignores the results, anesthetizes the dog, and performs the elective procedure. The dog does fine and returns much later for its annual visit, at which time the liver enzymes are still abnormal and even a little higher than before. Again, the abnormal results aren't investigated with additional diagnostics. This goes on and on. Ultimately, the dog is referred to us and we identify advanced copper storage disease upon liver biopsy. We see the liver enzyme abnormalities documented in the record but no indication that additional diagnostics were offered.

I would have anesthetized the dog and performed the procedure, but I'd have had the animal return sooner to recheck the liver enzyme activities. If the enzyme activities remained high, I would have 1) performed liver function tests (or at least offered the option), 2) explained what information I expected to obtain, and 3) offered imaging studies. If the liver function tests and imaging results were normal but the ALT

activity remained chronically high, I would have recommended a liver biopsy. Sometimes this process takes months before an owner is motivated to achieve a definitive diagnosis.

Hawthorne: Let's say I have a middle-aged dog with moderate dental disease that is receiving a nonsteroidal anti-inflammatory drug (NSAID) because he has trouble traversing stairs. Last year the dog's ALT activity was at the low end of the reference range. But the preanesthetic blood test results now show that the dog's ALT activity has increased twofold or threefold above the reference range. The increase could be related to the dog's dental disease or the drug.



"ALT is a very sensitive indicator of liver disease but not necessarily specific for a primary disease that we have to treat."

—David Twedt, DVM, DACVIM

The owner doesn't want to stop the NSAID because the dog has been acting like a puppy again. What would you do?

Twedt: I would take the dog off the drug, clean the teeth, and follow up. If the enzyme abnormalities resolve, then I'd pursue other options, such as chondroprotective agents or specialty diets. And if the enzymes remain abnormal, I'd pursue the liver.

Steiner: I agree, but I'd also make sure the owner is 100% comfortable

with doing the procedure now rather than waiting.

Center: I would stop the drug, initiate antibiotic therapy for 10 days, recheck the enzymes, and clean the teeth. You don't want to pursue liver disease before you've cleaned the teeth.

DeNicola: So when should practitioners automatically pursue liver function testing when ALT is increased? What about animals with no previous test results, no obvious dental disease or other clinical findings? Should those animals immediately undergo additional diagnostics?

Hawthorne: In animals with twofold to threefold ALT increases, I'd recheck them in three to four weeks. If the enzyme activities are persistently high—that red flag has gone up two or three times—practitioners need to pursue additional diagnostics, even in clinically normal animals.

Twedt: After practitioners have investigated drugs, alternative therapies, and other diseases, and if they're performing an ovariohysterectomy or other abdominal surgery on a patient with increased enzyme activity, they should always take a liver biopsy while they're in there.

Steiner: I perform additional diagnostics immediately in patients with a fivefold to sevenfold increase in ALT activity, even if they don't have clinical signs. But in cases with up to fivefold ALT elevations, I monitor them and add other tests as needed.

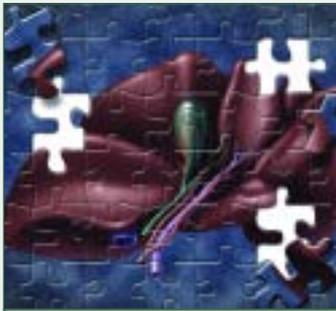
DeNicola: What is the half-life of ALT and how does it affect test interpretation?

Hawthorne: ALT activity seems to spike two to three days after a hepatic insult.

DeNicola: How long will ALT activity remain elevated? Or how often should we test to evaluate hepatocellular injury?

Center: An acute hepatic insult has ramifications beyond a single day. Hepatocytes adjacent to those undergoing cytolytic necrosis will also be damaged. Enzymes are liberated because of primary and secondary

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PUTTING THE PIECES TOGETHER:

- ALT elevations in dogs and cats primarily indicate hepatocellular damage.
- ALT activity seems to spike two to three days after a hepatic insult.
- ALT's half-life is very short, but you have to consider all the other things occurring in the liver, plus systemic disease and drug therapy.
- Two sources of ALKP are liver and bones.

phenomena. But if it's a one-time insult, the enzyme activities will decrease. For example, in dogs receiving NSAIDs, you may see ALT activities increase abruptly and then decline over a three-week period after drug withdrawal. The enzyme activity doesn't normalize as soon as the drug is withdrawn, which you would expect with a simple induction phenomenon.

While the rate of liver enzyme decline can be important over a relatively short time period, a decline in enzyme activity over a long time period can be misinterpreted. A substantial decline in functional hepatic mass may develop such that ongoing inflammation may not increase the enzyme activities as high as previously demonstrated. This scenario can be observed in dogs with cirrhosis where laboratory parameters may appear quiescent over six months or so. However, one day the dog may present for advancing ascites secondary to portal hypertension and hepatic fibrosis.

Twed: ALT's half-life is very short, but you have to consider all the other things occurring in the liver, plus systemic disease and drug therapy. In asymptomatic clinical cases with increased enzyme activities, I monitor the patient for several weeks. If enzymes do not return to normal in two to three weeks, I consider an ongoing insult, and that is a key to further investigating the patient.

Center: An abstract presented at an ACVIM conference details the half-life of injected transaminases. They were reported as 77 hours for ALT and 22 hours for aspartate aminotransferase (AST).⁴

Williams: So two to three days. We retest in intervals of weeks rather than days.

DeNicola: If we use the short half-life information regarding ALT, retesting before the three- to four-day period won't provide much information unless the patient has significant progressive disease.

Steiner: I wouldn't say that. If a dog comes in acutely sick with an ALT of 800 U/L, I want to know whether it's 300 or 3,000 U/L the next day.

Center: But checking them too frequently can also lead to mistakes. For exam-

ple, we see animals when they're desperately ill. They may be dehydrated, and we run the first blood sample collected. Then an intern loads the patient with fluids. The next morning, we collect a second blood sample, and the liver enzyme activities are decreased, but they're not in the normal range. They may be half what they were on the first test, but part of that is a dilution effect. So practitioners need to watch the trends but be aware of a dilutional phenomenon when rehydrating severely hemoconcentrated patients.

DeNicola: So generally speaking, much depends on the clinical presentation. The animals with active disease, primary or secondary, may need more frequent testing, while asymptomatic animals with increased liver enzyme activities may not need to be retested for several weeks to a month.

Alkaline phosphatase

DeNicola: Let's discuss the enzyme that's probably the most confusing to practitioners and clinical pathologists: ALKP. What sources of this enzyme should practitioners consider to be important?

Steiner: Two sources of ALKP are liver and bones. But in dogs, two isoenzymes originate from the liver: a liver-specific isoenzyme and a corticosteroid-induced isoenzyme.

Center: Studies show that the corticosteroid-induced isoenzyme in hepatocytes derives from the bile canaliculi (a specialized portion of the membrane between the hepatocytes) and the intestines.⁵⁻⁷ The important fact clinically is that this enzyme is induced before it increases in serum. This is why it reflects both cholestasis and induction.

DeNicola: What is the value of isoenzyme characterization?

Hawthorne: We look at increases in ALKP activity as a cholestatic problem. Or maybe it's induction. We've seen ALKP increases in some patients with inflammatory bowel disease, and we wonder why ALKP is elevated. It's probably coming from the gut, but we still look at that as a cholestatic mechanism.



DeNicola: It's a complicated issue because even with induction, such as corticosteroid induction, there's hepatopathy present as well, which results in cholestatic isozyme increases. At that point, the ALKP isoenzyme pattern becomes relatively insignificant to us.

At what point above the reference range do we need to categorize ALKP as cholestatic or induced?

Center: We've seen ALKP activities ten times higher (or more) than normal as an apparent result of induction.

DeNicola: But let's discuss lower increases. At what point can we eliminate bone and intestinal ALKP isoenzymes and consider cholestatic disease?

Center: I would be very surprised if bone produced more than a twofold increase.

Twedt: Two times. Maybe three, max.

DeNicola: That's what the literature says, so twofold to threefold is our cutoff point. But how specific is ALKP (in dogs) as a cholestatic disease indicator when ALKP activity is above that twofold to threefold increase?

Steiner: Of my canine patients with greater than fourfold ALKP elevations, about 80% have Cushing's disease, long-term corticosteroid administration, or some other condition—not cholestatic disease. So I would say 20% of dogs with fourfold ALKP elevations have cholestatic disease, but that's purely an estimate.

DeNicola: If we compare a dog with a greater than threefold ALKP increase to a dog with a greater than threefold increase in ALKP *plus* an ALT elevation, does that change your impression of what the ALKP increase means?

Steiner: If a dog has a threefold ALKP elevation and a 1.5-fold ALT elevation, the ALKP is the driving force behind my workup. But if both enzyme activities are increased threefold, then ALT becomes the driving force of my workup, and I search more intently for Cushing's disease than cholestatic disease.

Twedt: With increases in ALKP activity alone, I always think of corticosteroids, including topical medications, such as otic or ophthalmic preparations.

Center: Or homeopathic agents that contain adrenocorticotrophic hormones (ACTHs).

Twedt: My differential diagnoses for dogs with increased ALKP activities are Cushing's disease, hepatocellular adenomas, vacuolar hepatopathies, and possibly metastatic bone tumors. I often do imaging studies because I'm concerned about neoplasia in older dogs with elevated ALKP activity.



“Practitioners need to do more than a general blood chemistry profile to accurately assess liver function.”

—Dennis DeNicola, DVM, PhD, DACVP

DeNicola: What about dogs with high ALKP but no obvious signs of Cushing's disease—they're just drinking a little more. What's the next diagnostic step?

Center: We perform ultrasound-guided, fine-needle liver aspirates to see whether a vacuolar change is present. If we detect a vacuolar cytologic pattern, we sit down with the owner and discuss evaluating corticosteroid hormones, including a sex hormone profile. For us, it is more affordable to

discuss this option at the outset of testing so we can either 1) survey the sample for cortisol abnormalities, 2) store a portion of the sample for future sex hormone profiling (pre- and post-ACTH administration), or 3) pursue direct testing of cortisol and sex hormone values.

Steiner: I do the hormone profile, too. But I do an ACTH stimulation test first and obtain enough serum for the sex hormone profile as well. I measure cortisol concentrations first because it's an inexpensive test and I can do it in-house. If the ACTH stimulation test is normal, I send the samples for sex hormone testing.

Twedt: If I see a dog with a high ALKP and signs that may be compatible with Cushing's disease, I'll do an ACTH stimulation test. If the patient has a high ALKP and no signs that indicate Cushing's disease, I will do imaging studies to make sure I'm not dealing with morphologic liver changes. I have identified patients with liver neoplasia or other problems. I may do a fine-needle aspirate, and if the owner agrees, I'll measure the sex hormone concentrations. I find many of these vacuolar hepatopathies have increased 17-hydroxyprogesterone concentrations.

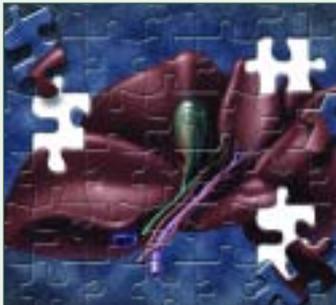
Hawthorne: Advanced diagnostic imaging is not a routine test for practitioners. The crux of diagnosing these liver diseases is and will be, for the foreseeable future, blood work—repeatable blood work and referrals. In my opinion, we don't do as many biopsies as we should. Twenty-five years ago when I started practicing, exploratory laparotomies were done all the time. We got lots of good answers, and now we're afraid to do them.

DeNicola: What do you do if a feline patient has increased ALKP activity?

Williams: I do additional workups to look for significant liver disease.

Twedt: I think it's unusual to see high ALKPs in cats, except for cats with hepatic lipidosis.

DIAGNOSING LIVER DISEASE



PUTTING THE PIECES TOGETHER:

- Increased ALKP is quite specific for cholestatic disease in cats.
- If AST increases occur in conjunction with marked ALT increases, that further confirms hepatocellular damage.
- Terrier breeds, including (but not limited to) Yorkshire terriers, bichons, Shih Tzus, Tibetan spaniels, and Havanese, may have abnormally increased serum bile acid concentrations because of microvascular dysplasia.

Center: Except we see high ALKPs in some cats with chronic cholangiohepatitis.

DeNicola: So increased ALKP is quite specific for cholestatic disease in cats. Much of this is based on the well-documented extremely short half-life of ALKP in cats from noncholestatic sources, such as bone and the gastrointestinal tract. This short half-life for ALKP in the cat is a double-edged sword because the half-life for cholestatic-source ALKP is also short. This makes ALKP extremely specific for cholestasis; however, because of the short half-life of the cholestatic source of ALKP, we see many cases of cholestasis in the cat without an increased ALKP. The sensitivity of ALKP for cholestasis in the cat is relatively low.

Gamma glutamyl transferase

DeNicola: Let's discuss GGT. In what tissues is it found, and which diseases cause GGT activity increases?

Twedt: GGT is found in the intestine, pancreas, liver, and kidneys. It indicates induction and cholestasis. I evaluate GGT more commonly in cats. When I'm trying to decide if a cat has biliary tract disease or hepatic lipidosis, I look at GGT in relationship to ALKP; ALKP may be higher than GGT activities in hepatic lipidosis. I've also had cases where GGT has been the major enzyme abnormality in dogs having significant cholestatic liver disease. So I don't discount GGT in dogs.

DeNicola: In the normal liver, ALKP is associated with the bile canalicular surface of the hepatocyte, but GGT is associated more with the biliary epithelial cells. In that regard, I look at the enzymes as a profile. GGT may not be associated with an active cholestatic process, but it may indicate biliary epithelial hyperplasia. How do the rest of our panel members use GGT?

Center: We evaluate GGT, which is cell membrane-affiliated, in dogs and cats. An early study we did showed that cats that did not have cholangiohepatitis or pancreatitis as primary disorders causing inappetence and hepatic lipidosis didn't develop GGT elevations.⁸ Consequently, GGT can be used as a flag to indicate inflammatory disease involving cell membranes in the

pancreas or liver. In some of our cats with hepatitis, GGT activity is higher than the coordinate ALKP activity when you look at the achieved fold increases, respectively.

DeNicola: I agree. If practitioners evaluate GGT, they must make sure they have an accurate reference range for the species they're dealing with.

Center: I would suggest that practitioners look at GGT activity in dogs with a hepatic mass. If GGT is increased, it may indicate a hepatocellular carcinoma, a malignancy that they'd want to investigate by obtaining thoracic radiographs and looking for metastatic disease.

Hawthorne: I agree. In that situation, the cost-benefit ratio of measuring the GGT is very good.

DeNicola: What about GGT half-life?

Center: I think the induction phenomenon underlying the enzyme increase makes it a moot point. Usually, the induction phenomenon abates slowly so that the serum enzyme activity diminishes slowly.

AST

DeNicola: I would like to briefly discuss AST. AST is relatively widely distributed in tissues not specific to the liver, making increases in enzyme activity difficult to interpret. Do any of you use it specifically for the liver?

Twedt: I associate increased AST activity with muscle and liver problems. First I look at creatine kinase (CK) activity. If this is high, I assume that the AST elevation indicates muscle damage. But I keep in mind that major muscle damage can cause ALT, AST, and muscle creatine kinase (CK₃) activity increases. However, if AST increases occur in conjunction with marked ALT increases, that supports hepatocellular damage. AST is found in mitochondria, and it is free in the cytosol. Marked increases of ALT and AST and normal CK suggest significant hepatocellular damage.

DeNicola: If you can eliminate muscle and erythrocyte origins (hemolysis), what does the AST and ALT profile tell you regarding their return to normal values?



Twedt: AST has a shorter half-life, so if you're following a case and see the AST returning to the normal range, that may be a better indicator of prognosis, especially in animals with acute liver toxicoses.

Center: I evaluate AST activity in dogs and cats. In animals with acute fulminant hepatic failure, the AST activities are often profoundly increased, possibly reflecting mitochondrial and more severe hepatocellular injury. No one has yet demonstrated the utility of the AST:ALT ratio as applied in human medicine where it implicates the severity of hepatocellular necrosis (*i.e.*, AST > ALT signifies mitochondrial injury).

DeNicola: Other liver enzymes, such as SDH and LDH, provide minimal value for the small animal practitioner. Practitioners won't get SDH values from in-clinic chemistry analyzers. Some doctors will get them from academic hospitals or reference labs. SDH actually proves to be relatively specific for hepatocellular injury and is helpful in ruminants and horses where ALT has little or no value.

LDH is available with some in-clinic chemistry analyzers and from reference laboratories. Veterinarians must keep in mind the wide distribution of LDH and be cautious in interpreting increased enzyme activity.

Liver function testing

DeNicola: Liver enzyme activities don't relay information about liver function. Elevations in these enzymes indicate only hepatocellular injury (ALT), induction or cholestasis (ALKP), and cholestasis (GGT). In addition, enzymes don't say anything about the reversibility or irreversibility of the damage. With that said, how do you define a function test?

Williams: A liver function test evaluates the overall functional capacity of one aspect of liver metabolic activity. And inadequate perfusion will also impair hepatic function.

Twedt: That's an important point because the liver has many different

functions, and there isn't one function test that evaluates all of them.

Hawthorne: The liver has more than 1,500 documented functions.

DeNicola: Traditionally, we sort function tests into five categories: hepatic synthesis, peripheral blood uptake, conjugation, secretion, and portal blood clearance. Is this reasonable for practitioners trying to decipher the severity of liver disease? Some of the function tests are included in our routine chemistry profiles, such as albumin, glucose, and cholesterol for synthesis and bilirubin profiles for peripheral blood uptake, conjugation, and secretion.



"If I have a feline patient with increased ALKP activity, I do additional workups to look for significant liver disease."

—David A. Williams, MA, VETMB, PhD, MRCVS, DACVIM, DECVM-CA

Center: But there are extenuating circumstances associated with all the parameters on a chemistry profile relating to liver function, which makes data interpretation a complex process. Glucose is a good example. Does the animal have sepsis? What's the insulin concentration? Is it a dog or a cat? How old is it? Does the patient have a high white blood cell count? Has the animal been receiving intravenous dextrose?

DeNicola: So even if you're addressing liver-specific parameters, you still have

to consider other variables and look at the whole animal.

Sensitivity of liver function tests

DeNicola: What is the sensitivity of the commonly used function tests for hepatic synthesis (albumin, glucose, and cholesterol) vs. hepatic uptake, conjugation, and secretion (bilirubin and bile acid profile)?

Steiner: By definition, they can't be sensitive because the liver has an overcapacity for almost anything it synthesizes. I don't think there's a single test that would show a change with less than 50% damage.

Twedt: But the sensitivity increases as you go down that list.

DeNicola: You're saying that tests like the bile acid profile are more sensitive than albumin, glucose, or cholesterol concentrations or bilirubin profiling.

Serum bile acid assays

DeNicola: Bile acid assays are sensitive indicators of hepatobiliary disease. When should bile acid assays be done?

Williams: If you measure bile acids only in animals with suspected liver disease, you can interpret the test results with confidence. If you measure bile acids routinely as part of a metabolic panel and not just in patients with suspected liver disease, a number of animals will have abnormal test results that are difficult to interpret because their clinical pictures do not fit with primary liver disease.

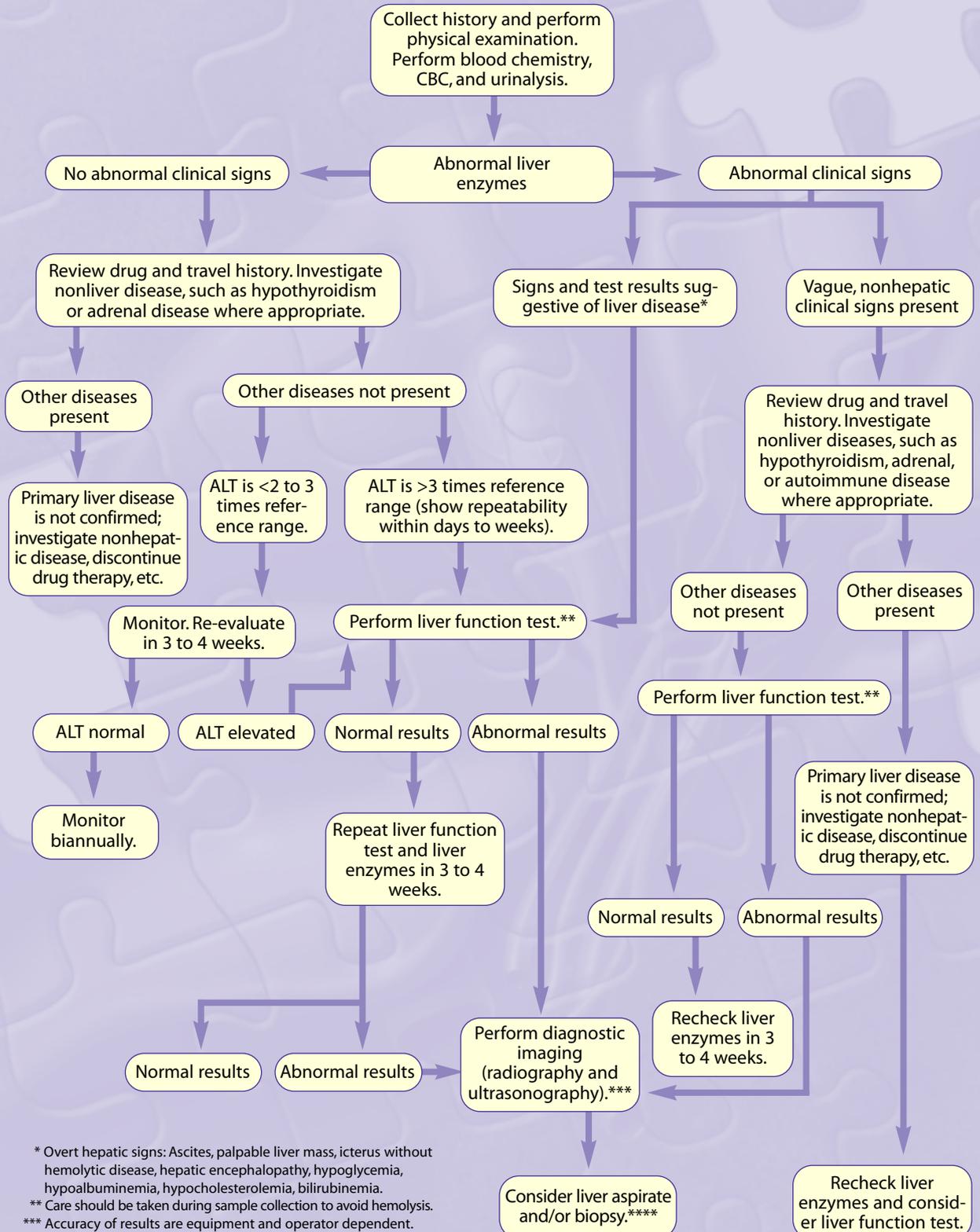
DeNicola: So do you choose to perform a bile acid assay based on clinical presentation or your enzyme panel results?

Williams: It depends on the whole clinical picture: the animal's clinical signs, the abnormalities that the chemistry panel does or doesn't reveal, and whether the patient is a young dog or cat.

Twedt: I measure bile acids if the liver enzyme activities are abnormal and I want to investigate the liver's function

DIAGNOSING LIVER DISEASE

The Steps in Investigating Liver Disease



* Overt hepatic signs: Ascites, palpable liver mass, icterus without hemolytic disease, hepatic encephalopathy, hypoglycemia, hypoalbuminemia, hypocholesterolemia, bilirubinemia.

** Care should be taken during sample collection to avoid hemolysis.

*** Accuracy of results are equipment and operator dependent.

**** Coagulation panel (PT, PTT, BMBT, platelet evaluation) should be completed before sample collection.



or if I suspect a vascular anomaly, such as a congenital portosystemic shunt. If I see an animal with obvious liver disease or a large, palpable liver mass, I wouldn't do a bile acid assay. I use bile acids to tell me if I should further investigate the liver.

DeNicola: So focusing on the group of animals with abnormal liver enzyme activities (vs. general screening) is probably most valuable.

Hawthorne: In our practice, we measure bile acids after obtaining a blood chemistry panel showing elevated liver enzyme activities but before performing imaging studies.

Twedt: Bile acid assays are relatively inexpensive, and they provide a lot of good information.

Center: There's just one problem, and it happens in terrier breeds, including (but not limited to) Yorkshire terriers, Shih Tzus, Maltese, bichons, Tibetan spaniels, and Havenese. Many of these breeds may have abnormally increased serum bile acid concentrations because of microvascular dysplasia.

Williams: And there could be other things going on as well, especially in Yorkshire terriers. They can have protein-losing enteropathy but not have major liver disease, and, in many cases, those small intestinal diseases are detected by observing panhypoproteinemia on blood chemistry panels.

DeNicola: How should practitioners perform total bile acid assays?

Williams: Practitioners need to conduct both fasting and post-feeding tests on every patient at the same visit. The timing for the post-feeding is two hours.

Hawthorne: Does the type of diet—low- or high-protein, low- or high-fat—affect the results?

Center: We compared different diets during development of the clinical serum bile acid test. Our findings served as the basis for recommending a maintenance canned-food product in

an attempt to standardize the testing procedure. The point is to feed a meal that will consistently stimulate enteric and gallbladder motility and produce an adequate challenge of the enterohepatic bile acid circulation.⁹ Table 2 on page 12 provides guidelines for bile acid testing.

DeNicola: What values should practitioners consider as important increases above the reference ranges?



“The liver has an overcapacity for almost anything it synthesizes. I don't think there's a single test that would show a change with less than 50% damage.”

—Jörg M. Steiner, M.D., D.A.C.V.I.M., D.E.C.V.I.M.-C.A.

Center: We set a range of up to 25 $\mu\text{mol/L}$ for dogs and up to 20 $\mu\text{mol/L}$ for cats.⁹ These values are based on a broad spectrum of samples from ill animals that were definitively shown not to have liver disease.

DeNicola: So the fasting and the postprandial samples should fall below that 20 to 25 $\mu\text{mol/L}$ range.

We've already said that bile acid assays are sensitive indicators of hepatobiliary disease, whether it's hepatocellular dysfunction, a vascular perfusion problem, or cholestatic disease. Is it possible to use serial bile acids to follow defined liver disease progression or regression?

Twedt: I use serial bile acid assays in dogs that have had shunt surgery to determine if the procedure was successful. With hepatocellular disease, it is common for bile acids to fluctuate from sample to sample with continued disease, but they will return to normal when the disease resolves.

Center: Bile acid results can widely vacillate with changes in intestinal motility and gastric emptying. Numerous physiologic variables influence this test.

Williams: We've done repeatability studies on unconjugated bile acids, and they are all highly variable when they are abnormally high. The concentrations are all abnormal, but the range from day to day or even hour to hour is huge. Post-feeding changes are also enormous. Total bile acids can also vary markedly, and it is not uncommon for fasting serum bile acids to be greater than the postprandial levels, probably reflecting contraction of the gallbladder in the fasting state.

DeNicola: Is there a situation where doctors shouldn't perform total bile acid assays?

Twedt: I wouldn't measure them if I know an animal has overt liver disease.

DeNicola: In that case, you would do the next logical diagnostic procedure, which is a biopsy.

Williams: The bottom line is that if practitioners obtain abnormal serum bile acid concentrations in patients that probably don't have liver disease (based on the entire clinical picture), they should consider malabsorption diseases, such as small intestinal disease and pancreatic insufficiency, which are often associated with changes in the intestinal microflora. It may or may not be appropriate to pursue possible liver disease further even though serum bile acids are increased. I wasn't aware of that three years ago, but I'm absolutely convinced of it now.

Plasma ammonia concentration

DeNicola: What about ammonia testing? The liver obviously plays a critical role

DIAGNOSING LIVER DISEASE

Table 2

Guidelines for Bile Acid Testing

1. Don't collect a blood sample and force it through a small needle into a Vacutainer. This will result in hemolysis and confuse result interpretation. Hemoglobin shares an absorption spectrum with the test endpoint dye, which obfuscates interpretation of the serum bile acid concentration.
2. Remove the needle from the collection syringe, and gently place the blood sample into a heparinized Vacutainer tube.
3. Don't submit samples in separator tubes; leakage of a lipemic sample will result in hemolysis that will contaminate the separated serum. It is better to centrifuge the sample, separate the serum or plasma, and specify ultracentrifugation for removal of potentially interfering lipemia. Lipemia substantially interferes with spectrophotometric endpoint detection. If these steps are taken, there will be less of a problem interpreting serum bile acid test results.

in metabolizing ammonia originating from protein metabolism; ammonia is processed to urea, which is then excreted through the kidneys. Should we be measuring resting (fasting) and postchallenge plasma ammonia levels to evaluate liver function?

Hawthorne: We do it as a screening test when we suspect hepatoencephalopathy.

Steiner: I use basal ammonia concentrations as an early monitoring tool in dogs with severe liver disease. If the ammonia concentration is elevated, I assume the dog has subclinical hepatic encephalopathy even if the dog doesn't show any neurologic signs. I'll treat for hepatic encephalopathy, and if the dog responds with better behavior or a better appetite, then I keep treating for it.

Center: I look for urine biurates (ammonium biurate crystalluria) in dogs with suspected portosystemic vascular anomalies. Ammonium biurate crystals are easy to recognize and indicate the presence of hyperammonemia. Detecting hyperammonemia does have value as nothing else we can measure correlates with hepatic encephalopathy better than ammonia. However, not all patients with portosystemic vascular anomalies are hyperammonemic. Nevertheless, many of them will demonstrate ammonium biurate crystalluria when several urine samples from different days are examined.

Twedt: We rarely perform ammonia testing; I don't use it as a diagnostic or screening test. But I measure baseline ammonia concentrations in cases where I suspect hepatic encephalopathy or observe signs of dementia or depression.

DeNicola: Is ammonia testing worthwhile in icteric dogs that have cholestasis?

Center: If they're symptomatic, I think you can get the same information by evaluating the urine carefully. In my opinion, veterinarians must be able to identify ammonium biurates because I think that some technicians reading urinalyses aren't sufficiently trained to detect these crystals.

DeNicola: I think we can summarize that plasma ammonia measurement is valuable for characterizing patients with hepatic

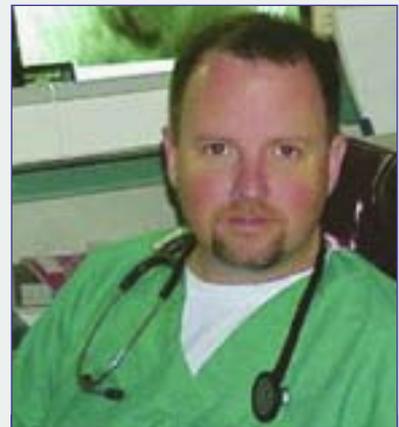
encephalopathy. Identifying ammonium biurate crystalluria, which is commonly seen with persistent hyperammonemia, may provide the same information.

Bilirubin

DeNicola: Total bilirubin concentration is widely accepted as being a valuable test, but what about conjugated and unconjugated bilirubin profiles?

Steiner: Research has shown that they aren't useful.^{10,11}

Center: We evaluate both profiles because we have the reagents, and the results are reported on our profiles. But the van den Bergh fractionation of total bilirubin into direct (conjugated) and indirect (unconjugated) moieties is not useful for achieving a definitive diagnosis.



"Digital radiography is tremendous because, unlike traditional radiography using film, you can make a technique error and still obtain a quality image, thanks to computer post-processing."

—Brian Poteet, MS, DVM, DACVR, DABSNM

Twedt: We don't include them.

DeNicola: What's your basic argument with them—the fact that it's 50-50 unconjugated and conjugated or that they don't help you determine if cholestasis is present?

Center: The conjugated bilirubin gets protein-bound.



Figure 1. A radiograph showing a small liver. The overall liver volume is decreased, and the stomach axis is deviated cranially.

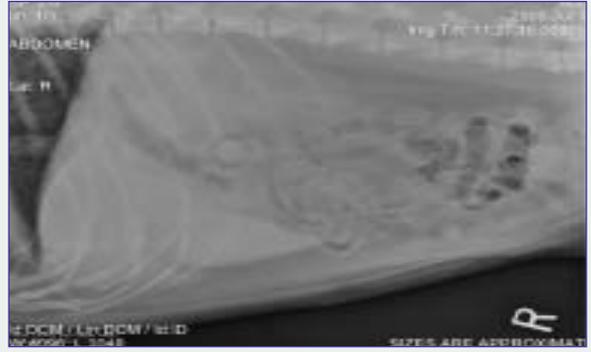


Figure 2. A radiograph showing a mildly enlarged liver. The liver extends beyond the costal arch, and the stomach axis is deviated caudodorsally. The caudal ventral aspect of the liver tapers normally.

Radiography

DeNicola: When should diagnostic imaging be used in evaluating liver disease or liver function in dogs and cats?

Dr. Brian Poteet: Anytime an animal has clinical signs that can be attributed to the liver or the biliary system and if the initial blood work shows elevated liver enzyme activities. Practitioners should start with radiographs of the abdomen because they provide information that can't always be obtained using ultrasonography. In our practice, we rarely perform ultrasonography without obtaining plain films first.

DeNicola: So what should practitioners be looking for primarily?

Poteet: They should first look for an enlarged or small liver, then for a liver with an irregular shape (Figures 1 and 2). Also, any decrease in abdominal serosal detail can indicate a modified transudate or blood leaking from the liver.

DeNicola: What about patient positioning?

Poteet: A lot of veterinarians, especially with dogs that weigh more than 40 or 50 lbs, will shoot one lateral and one ventrodorsal view and miss the cranial and caudal aspects of the abdomen. They need to radiograph the entire abdomen on both views. If they're looking at the liver and stomach, they need to see the entire liver.

Twedt: It's also important to investigate for other abdominal problems that could



“We measure bile acids after obtaining a blood chemistry panel showing elevated liver enzyme activities before performing imaging studies.”

—Robert Hawthorne, DVM

cause abnormal liver enzyme activities. I check for evidence of prostatic or renal disease and pyometra.

DeNicola: What about new technology in basic radiology?

Poteet: Digital radiography is tremendous because, unlike traditional radiography using film, you can make a technique error and still obtain a quality image, thanks to computer post-processing. You still need a cooperative animal that is positioned correctly, but digital radiography reduces the number of retakes and eliminates the need for processors and chemicals. The problem right now is cost. Digital radiography is

relatively expensive for many practitioners. But that should change over time as more doctors make the switch.

Ultrasonography

DeNicola: What protocol should practitioners follow when they perform liver ultrasonography?

Poteet: Perform a systematic and thorough abdominal evaluation every time, especially with deep-chested dogs. When evaluating the liver specifically, I usually start on the left side of the liver and advance to the right side with the dog in dorsal recumbency. I make sure that I see the entire liver and that I don't go through it so fast that I miss subtle nodules. In deep-chested dogs, you may not see everything underneath the diaphragm, so you may need to place the transducer in the intercostal spaces and clip a little more hair to evaluate that part of the liver. Also, I make sure I use the abdominal settings on the machine, not the cardiac settings.

DeNicola: How long does a complete ultrasonographic liver evaluation take?

Poteet: For general practitioners, I would say from five to eight minutes.

DeNicola: What information can practitioners gather from ultrasonographic liver evaluations?

Poteet: When I show practitioners how to perform liver ultrasonography, I begin by evaluating the intrahepatic vessels, the extrahepatic vessels, and the

DIAGNOSING LIVER DISEASE

Table 3

Possible Findings with Liver Ultrasonography

- fatty liver disease in cats
- nodular regeneration
- end-stage liver disease
- passive liver congestion
- liver abscesses
- benign cysts in the liver
- hepatocutaneous syndrome
- different types of neoplasms (hemangiosarcoma, adenocarcinoma, lymphosarcoma, mast cell disease)

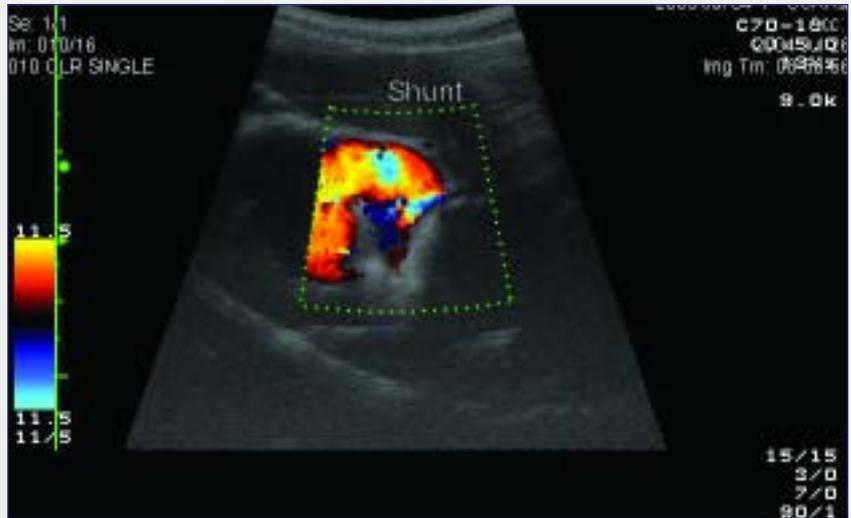


Figure 3. Static ultrasound image showing turbulent blood flow in a dilated and tortuous intrahepatic shunt.

porta hepatis, looking for evidence of any abnormal vascular structures and evaluating the normal structures for laminar blood flow (Figure 3). Then I evaluate the gallbladder and bile ducts for dilatation. Finally, I look at the liver itself and try to rule out common problems (Table 3).

DeNicola: Is ultrasonography helpful in cases of steroid- or sex-hormone-induced vacuolar hepatopathy?

Poteet: Most of the animals with vacuolar hepatopathy have diffusely hyperechoic livers. Ultrasonography is a sensitive test, but it also lacks specificity. The only way to confirm a diagnosis is through a fine-needle aspirate or a biopsy, but I think ultrasonography can help.

Center: What landmarks do you use when you're performing a vascular liver evaluation?

Poteet: First, I look at the caudal vena cava where it passes underneath the liver dorsally. Then I look for the portal vein and the porta hepatis as it enters the liver and try to follow the branches into the liver periphery.

Inside the liver, I look for the hyperechoic walls that are usually associated with the portal veins in the periphery of the liver; the hepatic veins don't have those. Unless there's a lot of free fluid in the abdomen, ultrasonography doesn't differentiate liver lobes very well.

Center: What size comparisons do you make to determine whether the vasculature within the liver at the porta hepatis is normal?

Poteet: There are no standards for hepatic vein diameter as it enters the liver. I think that determination is based on experience. The portal vein and the caudal vena cava should be similar in size, but there's a lot of variation. The caudal vena cava itself varies in size with the animal's respiration.

Center: I don't think most practitioners can do the vascular evaluations that you can do. I have many referrals where practitioners have tried, but they end up with incorrect diagnoses for the vascular group of diseases.

Poteet: That's probably true. We don't use ultrasonography to find exactly where single-vessel, extrahepatic shunts are because it takes me 30 to 45 minutes to do it. But in rare cases, you can find multivessel, extrahepatic shunts in dogs fairly quickly.

Advanced diagnostics

DeNicola: When should practitioners refer a case for advanced diagnostics, such as computed tomography (CT) or magnetic resonance imaging (MRI)?

Poteet: CT should be performed on all large-breed dogs suspected of having an



intrahepatic shunt. It's wonderful because you can use contrast to determine specifically where the shunt is located. When we suspect a portosystemic shunt based on clinical signs and elevated pre- and postprandial bile acids, we perform scintigraphy (portal scans). Scintigraphy can also help differentiate a macroscopic shunt from microvascular dysplasia.

We rarely perform MRIs for liver disease. At this point, I think that liver MRIs are unexplored in veterinary medicine. Abdominal CTs are really quick compared with MRIs.

Monitoring patients with liver disease

DeNicola: Dr. Poteet, do you use imaging to monitor liver disease in patients?

Poteet: Probably not as much as I should. We use imaging to monitor the numbers of nodules in oncology patients. Part of the decision comes down to pet owner finances. If imaging isn't going to change the animal's treatment, then it may not be worth it financially.

DeNicola: We've already discussed the frequency of measuring liver enzyme activities. In general, practitioners don't need to monitor liver enzymes daily except in progressive cases. With stable diseases, several days to a week between testing will help predict the general course of the disease. Is there anything else practitioners should do?

Center: I recommend a laparoscopic liver biopsy and another biopsy six months to a year later to evaluate the disease progression. Either way, a change in medication may be warranted.

Another important step that practitioners should remember is recording body condition scores. Record a quantitative score because it reflects the pet's health.

Twedt: Proper monitoring depends on the disease you're dealing with. If you're talking about chronic biliary tract diseases in cats or chronic hepatitis in dogs with copper storage disease, you need to rebiopsy to determine the patient's response to therapy. I often reevaluate chronic hepatitis cases with liver biopsies at six-month to one-year intervals. With acute disease, monitor the liver enzyme activities, make sure they return to normal, and make sure the animals are doing well clinically.

As far as monitoring response to therapy, you can't use laboratory tests to determine if dogs receiving corticosteroids for inflammatory liver disease are getting better or not. In these cases, a liver biopsy is often required.

DeNicola: I want to thank the participants in this roundtable discussion. Their expertise in the area of liver and gastrointestinal disease is invaluable, and hopefully the information that has surfaced today will aid veterinarians in their day-to-day evaluation of patients with liver disease.

It's clear that we must utilize our various diagnostic modalities, including clinical chemistry testing and diagnostic imaging, in conjunction with important information such as the age, breed, sex, and physical condition of animals with suspected liver disease.

Veterinarians must keep in mind that serial evaluation of the complete blood count and urinalysis findings add to the overall interpretation of liver disease diagnosis, progression, and regression. Also, serial evaluations of laboratory data and diagnostic imaging evaluations in patients with confirmed liver disease can be extremely helpful in characterizing the development of disease as well as the patient's response to therapy.

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Cover art by Paul Chason.

Radiographs courtesy of Brian Poteet, MS, DVM, DACVR, DABSNM; Gulf Coast Veterinary Diagnostic Imaging; Gulf Coast Veterinary Specialists, Houston, Texas
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