ABNORMAL LIVER ENZYMES: A PRACTICAL CLINICAL APPROACH

David C. Twedt, DVM, Diplomate ACVIM
Colorado State University
twedt@colostate.edu

The detection of abnormal liver biochemical tests in the asymptomatic as well as the symptomatic patient is a common finding on a routine blood screen. In humans it is reported that up to 4% of asymptomatic persons have increased serum liver enzymes. In a study of 1,022 blood samples taken from both healthy and sick dogs and cats 39% had ALP increases and 17% had ALT increases. The identification of liver biochemical abnormalities should suggest certain diagnostic possibilities and should guide a protocol for further investigation. Liver biochemical abnormalities are often nonspecific; the measured enzymes can be isoenzymes from another tissue or the same enzyme from a different tissue source. An understanding of the liver biochemical tests is essential when evaluating the patient in question. Liver biochemical test abnormalities are categorized into groups that reflect 1) hepatocellular injury, 2) cholestasis or 3) tests of impaired metabolic function or synthetic capacity.

**Laboratory Tests**

It is important to understand basic liver related laboratory tests in order to determine the possible etiologies for abnormal levels and to develop a course of action. Many liver tests are not specific only to the liver but can be abnormal from primary non-hepatic disease as well. Evaluation of liver biochemical tests must be interpreted in light of the history, medications and clinical findings. The magnitude and duration of increase is also dependent on the type, severity and duration of the stimulus and the species. They do not prognosticate the irreversibility of liver disease at one point in time. Also because the liver is involved in so many functions no single laboratory test in this category reflects the complete functional state of the liver.

**Indicator of Hepatocellular Injury.** A common presentation is the isolated increase in either alanine aminotransferase (ALT) or aspartate aminotransferase activity (AST). Canine and feline hepatocyte cytoplasm is rich in ALT and contains lesser amounts of AST. Altered permeability of the hepatocellular membrane caused by injury or a metabolic disturbance results in a release of this soluble enzyme. Conceptually ALT and AST should be thought of as hepatocellular "leakage" enzymes. Subsequent to an acute diffuse injury, the magnitude of increase crudely reflects the number of affected hepatocytes. It is however neither specific for the cause of liver disease or predictive of the outcome. The plasma half-life of ALT activity is 2.5 days and AST about 1 day, however ALT concentrations may take many days to decrease following an acute insult (possibly 2 weeks or longer). ALT elevations greater than 2X normal or persistent increases should be investigated. ALT increases are characteristic of chronic hepatitis in the dog.

**Specific Evaluation of Increased ALT.** Persistent ALT increases should be investigated or when they are greater than twice normal. The most important diagnosis to make is chronic hepatitis. Early diagnosis and prompt therapy improves patient survival. Hepatitis often begins in dogs 2-5 years of age with only ALT increases. Females are over-represented and breed associated hepatitis is well known. Dogs with significant hepatitis usually also have concurrent bile acid abnormalities. ALT elevations in young dogs under 1 year of age is sometimes associated with portal vascular anomalies and bile acid concentrations should be obtained to exclude that possibility. Occasionally I see young dogs evaluated prior to elective surgery having unexplained ALT increases for unknown reason that correct over time. Occasionally we will see dogs with mild increases in ALT and no histological evidence of disease and this probably reflects mild membrane changes not seen with light microscopy.

A variety of tissues, notably skeletal muscle and liver, contain high aspartate aminotransferase activity (AST). Hepatic AST is located predominately in hepatocyte mitochondria (80%) but also soluble in the cytoplasm. Skeletal muscle inflammation invariably causes a serum AST increase (and ALT to a much lesser extent) that exceeds the serum ALT activity and can be further defined as muscle origin by the measurement of the serum creatine kinase activity (CK) a specific muscle enzyme. Clinical experience in veterinary medicine indicates that there is value in the interpretation of the serum activities of ALT and AST for liver disease. Following an acute injury resulting in a moderate to marked increase in the serum ALT and AST concentrations, the serum AST will return to normal more rapidly (hours to days) than the serum ALT (days) due to their difference in plasma half-life and cellular location. By determining these values every few days
following an acute insult, a sequential “biochemical picture” indicative of resolution or persistent pathology is obtained.

**Markers of Cholestasis and Drug-Induction.** Alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) show minimal activity in normal hepatic tissue but can become markedly increased in the serum subsequent to increased enzyme production stimulated by either impaired bile flow or drug-induction. These enzymes have a membrane bound location at the canalicular surface; ALP associated with the canalicular membrane and GGT associated with epithelial cells comprising the bile ductular system. With cholestasis, surface tension in the canaliculi and bile ductules increases and these surface enzymes are then up-regulated in production.

**Specific Evaluation of Increased ALP.** Elevations in only ALP in the dog is a common observation. It has a high sensitivity (80%) but a low specificity (51%). This is because of the multiple isoenzymes of ALP that can be induced into production. Alkaline phosphatase is present in a number of tissues but only two are diagnostically important, bone and liver. The plasma half-life for hepatic ALP in the dog is around 70 hours in contrast to 6 hours for the cat and the magnitude of enzyme increase (presumably a reflection of the synthetic capacity) is greater for the dog than the cat. Bone source from osteoblastic activity occurs in young growing dogs before their epiphyseal plates close or in bone lesions (ie osteogenic sarcoma). In the adult dog without bone disease, an increased serum ALP activity is usually of hepatobiliary origin. However a recent study identified some dogs with osteogenic bone tumors to have increased ALP concentrations. ALP increase in those dogs indicates a poorer prognosis suggesting probable diffuse bone metastasis. GGT is not found in bones.

An increase in the serum ALP and GGT activity can also be associated with of glucocorticoids (endogenous, topical or systemic), anticonvulsant medications and possibly other drugs or herbs in the dog. There is remarkable individual variation in the magnitude of these increases and there is no concomitant hyperbilirubinemia. A moderate to marked increase in serum ALP activity without concurrent hyperbilirubinemia is most compatible with drug-induction and warrants a review of the patient’s history (topical or systemic glucocorticoids) or evaluation of adrenal function. The increased ALP has long been attributed to a glucocorticoid-stimulated production of a novel ALP isoenzyme in the dog that can be distinguished from the cholestatically-induced hepatic ALP isoenzyme by several procedures. It was initially thought that the glucocorticoid-associated isoenzyme could be used as a marker of exogenously administered corticosteroids or increased production of endogenous glucocorticoids. The glucocorticoid-associated isoenzyme has a very high sensitivity in animals with Cushing’s disease but a low specificity. Unfortunately, the glucocorticoid-associated isoenzyme is also associated with hepatobiliary disease as well.

A common condition observed in older dogs is an idiopathic vacuolar hepatopathy associated with increased ALP steroid isoenzyme. Investigation for hyperadrenocorticism is negative in these cases. It is postulated that other adrenal steroids may also be responsible in some cases. It is known that progesterones bind to the hepatocyte corticosteroid receptors and can induce ALP production. Scottish Terriers also have yet unexplained increases in serum ALP concentrations causing an idiopathic hyperalkalinephatasemia. Lastly, hepatic neoplasia and benign hepatic nodular hyperplasia both are sometimes associated with only ALP increases. Abdominal ultrasound should be performed to rule out neoplasia such as hepatic adenoma, adenocarcinoma or bile duct carcinoma. Multiple hyper or hypoechocic nodules in the liver of older asymptomatic dogs suggests nodular hyperplasia but wedge biopsy confirmation is advised.

Increased serum GGT activity is associated with impaired bile flow in the dog and cat and glucocorticoid administration in the dog. Bone does not contain GGT, therefore growth and bone disease are not associated with increased serum GGT activity. The administration of anticonvulsant medications to dogs is reported not cause an increase in the serum GGT activity. Colostrum and milk have high GGT activity and nursing animals develop increased serum GGT activity. As a marker of hepatobiliary disease, the measurement of serum GGT activity does not appear to provide a diagnostic advantage over the serum ALP determination in the dog, however it is reported to be a more sensitive marker in cats having biliary tract disease.

**Evaluation of Liver Function.** On a routine biochemical profile it is important to note the liver function tests including bilirubin, albumin, glucose, BUN, and cholesterol. Albumin is exclusively made in the liver and if not lost, sequestered or diluted, a low concentration would suggest significant hepatic dysfunction. It may take greater than 60% hepatic dysfunction for albumin concentrations to decline. Major clotting factors are also made in the liver (except factor 8).
therefore prolonged clotting time suggests hepatic dysfunction. Liver disease and abnormal function tests suggests hepatic failure and a guarded prognosis.

One of the most sensitive function tests we have readily available in small animals are serum bile acids. The fasting serum total bile acid concentration (FSBA) is a reflection of the efficiency and integrity of the enterohepatic circulation. Pathology of the hepatobiliary system or the portal circulation results in an increased FSBA prior to the development of hyperbilirubinemia, negating its usefulness in the icteric patient. An increase is not specific for a particular type of pathologic process but is associated with a variety of hepatic insults and abnormalities of the portal circulation.

The current suggestion for the determination of the FSBA is to differentiate between congenital portal vascular anomalies and liver insufficiency prior to the development of jaundice. The determination of FSBA can contribute to the decision to obtain histological support for the diagnosis of this last group of hepatic diseases. When the fasting value is greater than 25 µmol/L for the dog and cat, there is a high probability that the histology findings will define a lesion. When the FSBA concentration is normal or in the “gray zone” the FSBA should be followed by a 2-hour postprandial serum total bile acid (PPSBA) looking for an increase greater than 25 µmol/L. The diagnostic value of determining PPSBA concentration is increased sensitivity for the detection of hepatic disease and congenital portal vascular anomalies. When using these guidelines it is prudent to recognize that a small number of healthy dogs have been reported with PPSBA values above 25 umol/L and dogs with mild liver disease may have normal bile acids.

We have occasionally observed the measurement of a FSBA value higher than the PPSBA value. The reason for this non sequitur is unclear but probably multifactorial. It has been shown that (1) the peak PPSBA concentration for individual dogs is variable, (2) fasted dogs store about 40% of the newly produced bile in the gallbladder and (3) a meal stimulates the release of only between 5 to 65% gallbladder bile. Undoubtedly these physiologic variables in addition to physiological variation in intestinal transit time and concurrent underlying intestinal disease contribute to the dichotomy.

Recently, urinary bile acids have become available as a diagnostic tool. Identifying increased urinary bile acids provides similar information to what is obtained from serum bile acids and neither test appears to be better than the other. The advantage of urinary bile acid measurements would be for the screening of litters of young puppies for suspected inherited vascular anomalies where urine collection is simpler than paired serum samples.

In summary, there are a variety of markers with variable sensitivity and specificity that reflect hepatic tissue and portal vasculature pathophysiology. We support the conclusion of another study that found that the optimal test combination is the serum ALT activity and bile acid concentrations. This pairing provided the best sensitivity and specificity, respectively. Clinical experience indicates that elevated serum AST concentration along with an elevated ALT helps to support a diagnosis of hepatocellular disease and that the PPSBA concentration enhances the evaluation of hepatic function.

Management Strategies
In the asymptomatic patient with an increased liver biochemical test(s) the increased value should be confirmed at least once to exclude a spurious result from laboratory error and to avoid unnecessary and costly additional testing. A careful history is essential to exclude drug associated enzyme elevations. The signalment of the patient may also provide an insight to the possible etiology of the enzyme increase. For example old dogs frequently have benign nodular hyperplasia, neoplasia or systemic disease while younger to middle aged dogs more commonly have chronic hepatitis. There are also certain breeds that are predisposed to developing chronic hepatitis. A careful physical examination may also provide clues to the diagnosis. The most common cause of abnormal liver enzymes is not primary liver disease but rather reactive hepatic changes occurring secondary to other non-hepatic diseases. These would include such conditions as intra-abdominal disorders (IBD, nutritional abnormalities), cardiovascular disease or metabolic derangements as just a few examples. Generally these secondary changes are reversible once the primarily disease is treated. Successful resolution of the non-hepatic disease and continued abnormal liver enzymes would be a strong indication for further investigation of the liver.

If no likely explanation for the laboratory abnormalities can be found there are two courses of action that one can take; either begin a diagnostic evaluation of the patient starting with bile acid determinations, or re-evaluate the patient’s liver enzymes at a later date. A rational wait period for re-evaluation is 4-6 weeks giving consideration to the half-life of liver enzymes and the
time needed for recovery from an acute occult hepatic injury. It is best not to delay retesting beyond 6-8 weeks in the event that an active disease process may progress.

**Diagnostic Strategies**

Once further work up has been elected, if the patient is not icteric, the next diagnostic step should be evaluation of urine or serum bile acids. Abnormal bile acids indicate hepatic or circulatory abnormalities and that the patient should undergo further evaluation at this time.

**Imaging.** Routine abdominal radiographs are helpful in determining liver size and shape and for detection of other intra-abdominal disorders. Ultrasonography is noninvasive, readily available and is the most informative initial imaging modality for liver disease. It often complements the clinical and laboratory findings and is useful for identifying focal liver lesions, diffuse liver disease or biliary disease. Frequently, fine needle aspiration (FNA) for cytological evaluation is performed in conjunction with ultrasound. Although FNA is safe and easy to perform, one must be cautious in the interpretation of the results and use the FNA findings in conjunction with clinical signs and other diagnostics to make a diagnosis. The sensitivity and specificity is not very high when results are compared to histopathology. We find the best correlation with hepatic neoplasia and diffuse vacuolar hepatopathies and the poorest in patients with chronic hepatitis.

**Liver Biopsy.** Although our diagnostic techniques continue to improve, in most instances imaging and biochemical testing cannot replace a liver biopsy. This is by far the best examination for a definitive determination of the nature and extent of hepatic damage and to appropriately direct the course of treatment. The method for liver biopsy procurement may be surgery, needle biopsy or laparoscopy. Each has certain advantages and disadvantages and the decision of which procedure to use should be made in light of all the other diagnostic information, always considering what is in the best interest of the patient and client.

**Summary**

Abnormal liver enzymes should not be ignored and should be investigated in a systematic manner as previously discussed. Asymptomatic animals with no evidence of significant or treatable disease or in situations where financial constraints limit further work up the patient should be fed a quality maintenance diet for the patient’s stage of life and the possibility of instituting specific liver support therapy should be explored.

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**ABNORMAL LIVER ENZYMES**

- **Asymptomatic**
  - Consider antibiotics +/- liver support
  - Repeat liver enzymes in 1-2 months and/or bile acids
  - If abnormal
- **Symptomatic**
- **Obvious primary liver disease**
- **Look for a primary non-hepatic disease or drug history**
  - Treat primary non-hepatic disease
  - Re-evaluate liver
- **Work up the liver**

**Reactive Hepatopathies**

The so-called “reactive hepatopathies” which occur secondary to non-hepatic disease can result in increased serum biochemical hepatic tests and histomorphologic abnormalities. Most of
the reactive hepatopathies cause increases in laboratory tests that evaluate hepatocellular integrity (ALT, AST) and tests of hepatic cholestasis (ALP, GGT). In most cases there are little if any changes in tests that evaluate hepatic function (bilirubin, albumin, glucose, and BUN). Most of the animals with secondary liver disease also retain normal serum bile acid concentrations, which again supports a concept that there is generally minimal hepatocellular dysfunction in most of these disease conditions.

This group is characterized by nonspecific hepatocellular degeneration or necrotic changes without evidence of significant chronic progressive inflammation. Again, these changes are usually secondary to manifestations of a primary non-hepatic disease. The reason the liver often undergoes these changes revolves from the fact that the liver is involved in many metabolic and detoxification functions. Endogenous toxins, anoxia, metabolic changes, nutritional changes and endogenous stress related glucocorticoid release are all examples of conditions responsible for the majority of these changes. Non-specific mild liver changes routinely also occur following general anesthesia.

A good example that helps explain this concept is inflammatory bowel disease in which it is not unusual to observe mild inflammatory changes around portal triads presumed to be the result of abnormal portal uptake of gastrointestinal “toxins”. Throughout the liver and closely associated with portal areas are Kupffer cells (fixed macrophages) that function to filter the blood of injurious toxins, inflammatory mediators and bacteria. When this macrophage system is abnormally insulted Kupffer cells release their own inflammatory mediators that in turn insult the hepatocytes.

Another example could be the sick septic dog having vacuolar change thought to be due to endogenous cortisol release for endogenous stress and hepatic cholestasis from presumed endotoxin or cytokine alteration of bilirubin metabolism.

Histological findings associated with secondary reactive changes include descriptors such as vacuolar degeneration, hydropic degeneration, swollen hepatocytes, lipidosis, intracellular or intrahepatic cholestasis, mild multifocal hepatitis and periportal or variable hepatic necrosis. These changes are devoid of the typical progressive chronic inflammatory cell infiltrates characteristic of chronic hepatitis.

In a review of consecutive liver biopsies at Colorado State University histology grouped as non-specific reactive changes made up the largest category of abnormalities (approximately 25%) In this group we were able to identify an associated disease in many that could explain the likely cause for the hepatic enzyme increases and histological changes observed. Concurrent diseases identified included neoplasia, gastrointestinal, renal, autoimmune, dermatologic, dental, infectious and cardiac disease as a few examples. In some cases an underlying disease is not identified. The ALT values on the average are 1-2 X normal and the ALP values 1-3 X normal. It is interesting to note that in a series of 32 dogs having reactive hepatopathies, 8/8 cases in which serum bile acids were run, all were within the normal reference range again suggesting hepatic function remains intact.

This category appears to be the most common histological change to occur in dogs and is by far the most common cause of elevated liver enzymes. Based on this fact, dogs presented with elevations in ALT and ALP should always have primary non-hepatic disease ruled out first. These changes are usually very reversible and no specific hepatic therapy is required short of treating the primary disease. The liver changes resolve once the primary etiology is successfully treated. Therapy providing good liver support such as antioxidants may be warranted.