WEB CHAPTER 50
Evaluation of Elevated Serum Alkaline Phosphatase in Dogs

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Increased serum alkaline phosphatase (ALP) is a common laboratory finding in canine patients. In one survey of consecutive blood samples submitted to a reference laboratory, 39% of all dogs and 51% of dogs older than 8 years of age had increased ALP levels (Comazzi et al, 2004). The high sensitivity (86%) of increased ALP for detection of liver disease is complicated by the poor specificity (49%) because numerous diseases outside of the liver, as well as drugs and glucocorticoids, may induce production of the enzyme. Often the evaluation of canine patients with increased ALP becomes a diagnostic dilemma.

Pathophysiology

ALP is a heterogeneous group of enzymes with poorly defined biologic functions that catalyze the hydrolysis of phosphate from various organic compounds at an alkaline pH. Normally bound to cellular membranes, ALP has been isolated from the kidney, liver, bone, placenta, and intestine. Two genes encode for ALP: tissue nonspecific (TNS) and intestinal. The products of these genes are two isoenzyme proteins with different polypeptide sequences but similar enzymatic function. Products of the TNS gene include the isoforms of ALP found in the kidney, liver, bone, and placenta; and each differs only in degree of glycosylation. The intestinal gene encodes for the ALP found in the intestine (I-ALP), as well as corticosteroid ALP (C-ALP), a unique enzyme found only in the dog. Although several different isoenzymes/isoforms of ALP have been identified, only three are measured in serum because of their longer half-lives (approximately 70 hours): liver (L-ALP), bone (B-ALP), and C-ALP. The half-life of I-ALP, kidney, and placenta ALP is minutes.

Total serum ALP is the sum of L-ALP, B-ALP, and C-ALP. The proportion of each isoenzyme changes with age in normal dogs. B-ALP predominates in dogs less than 1 year of age, making up 96% of the total ALP; this proportion declines with age to approximately 25% in dogs older than 8 years of age. C-ALP makes up 10% to 30% of ALP in normal dogs, with higher proportions present in older dogs and smaller proportions in younger dogs. L-ALP is the predominant isoenzyme in dogs older than 1 year of age.
Liver Alkaline Phosphatase

L-ALP is located predominantly in the perilobular zone of the liver associated with hepatocyte membranes that compose the bile canaliculi and the sinusoidal membranes. Two mechanisms are responsible for increasing serum activity of this enzyme: cholestasis and drug induction. Induction is the increased synthesis of a protein or enzyme through modification of transcription, translation, or other processes involved in protein synthesis. Cholestasis leads to accumulation of bile salts from impaired bile flow and induces the production of L-ALP. The enzymes then accumulate on hepatocyte membranes, and solubilization of the membrane-bound enzyme occurs by the activity of glycosylphosphatidylinositol (GPI) phospholipase, an enzyme found in the plasma and on cellular membranes. Bile salts have been shown to play an important facilitatory role by increasing activity of GPI phospholipase and thus releasing L-ALP into circulation.

L-ALP induction also occurs secondary to various drugs, most notably phenobarbital and endogenous or exogenous glucocorticoids. The underlying pathophysiological mechanisms are poorly understood. Elevated L-ALP, B-ALP, and C-ALP were found in one study after phenobarbital administration, finding most consistent with induction because all isoenzymes were affected (Gaskill et al, 2004). However, differences in total L-ALP were not found between treated and untreated dogs in a separate study, an unexpected finding if induction was the primary mechanism (Gaskill et al, 2005). Increased serum levels may be secondary to increased activity of GPI phospholipase and subsequent release of ALP in serum or by direct liver injury, causing cholestasis. Glucocorticoids have been shown to increase serum C-ALP and L-ALP through induction by increasing levels of messenger ribonucleic acid (mRNA) within 24 to 48 hours of exposure.

Corticosteroid Alkaline Phosphatase

C-ALP is a product of the I-ALP gene that differs from I-ALP only in carbohydrate composition. I-ALP is devoid of carbohydrates, whereas C-ALP is glycosylated heavily with sialic acid and is thought to be responsible for the different half-lives of the two enzymes. The hepatocyte is the site of de novo synthesis of C-ALP after exposure to endogenous and exogenous glucocorticoids (Wiedmeyer et al, 2002). Expression of the I-ALP gene in the liver is delayed after exposure to steroids, as shown by increased mRNA expression and elevated L-ALP and serum C-ALP approximately 10 days after initiation of prednisone in experimental dogs. C-ALP accumulates on hepatocyte membranes in the area that composes the bile canaliculi and sinusoidal surfaces, and elevated serum levels occur through solubilization of C-ALP by activity of GPI phospholipase.

Total ALP can be quantified further by determining the percent of C-ALP isoenzyme in the serum using electrophoresis, selective inhibition (levamisole treatment), or a heat inactivation process in the laboratory. Measuring the isoenzymes of ALP is an available assessment in most diagnostic laboratories but often is of little clinical use. For example, the high sensitivity (95%) of elevated C-ALP for detection of hyperadrenocorticism (HAC) is complicated by the poor specificity (18%). Elevations in C-ALP are often present with diabetes mellitus (DM), primary liver disease, or other chronic illness possibly because of stress and increases in endogenous cortisol. Although finding increased C-ALP cannot confirm the presence of HAC, it generally is accepted that low values of C-ALP decrease the likelihood of this disease. Progesterones also are thought to bind to the corticosteroid receptor on the hepatocyte and may induce C-ALP production; however, this has been more recently questioned (see Chapters 50 and 148). Phenobarbital has also been shown to induce production of the C-ALP isoenzyme through unknown mechanisms.

Bone Alkaline Phosphatase

B-ALP is attached to the external cellular membrane of osteoblasts. The function of the enzyme is unknown, but it likely plays a role in bone formation. Increased serum levels generally are associated with increased osteoblastic activity that occurs with bone growth in young animals, fracture healing, osteosarcoma, and less commonly, nutritional osteopathies and renal secondary hyperparathyroidism. Elevations are typically mild (<4× normal) with osteosarcoma; however, they have been correlated to poor survival and are found only in tumors with increased osteoblastic activity (i.e., osteoblastic osteosarcoma). Benign familial hyperphosphatasemia has been reported in Siberian huskies and results from increases in B-ALP.

Diagnostic Evaluation

History and Physical Examination

Evaluation of the patient with elevated ALP begins with a thorough history and physical examination (Web Figure S50-1). The signalment may provide insight as to the underlying cause of ALP elevation. For instance, mild elevations in ALP (<2× normal) often are present in young, growing animals because of increased B-ALP. Often the calcium and phosphorous also are increased mildly in these dogs, and all three are expected findings in young dogs. Postsuckling puppies also have increases in ALP, possibly as a result of colostrum ALP or induction of ALP after ingestion of colostrum. Values typically return to normal within 10 days.

Because elevated ALP may occur as a result of enzyme induction, a careful drug history should be obtained. Commonly identified drugs include glucocorticoids and phenobarbital. In addition to oral steroids, topical and oculaur forms also may be associated with increased ALP. Specific questioning usually is needed to ensure that other forms of steroids are not being given because topical therapy often is not considered a medication by owners and frequently is not disclosed without direct questioning. Exposure to phenobarbital or other anticonvulsants often is determined easily, but other drugs also have been associated with elevated liver enzymes and liver toxicity and are discussed elsewhere (see Chapter 140). Various
Gastrointestinal Diseases

Higher elevations in ALP may be seen from DM alone, but concurrent diseases also should be considered if this is the case (i.e., pancreatitis, neoplasia, primary liver disease, or HAC). Similarly ALP occasionally is increased in hypothyroid dogs, but this finding is inconsistent. Abnormal concentrations of progestins (progesterone or 17-hydroxyprogesterone) also are thought to be associated with increases in serum ALP in some dogs.

Preliminary Evaluation
When an elevation in serum ALP is identified, routine blood work, including complete blood count, a comprehensive serum biochemistry, and urinalysis, should be performed. Primary hepatic disease, secondary reactive hepatopathies, and induction caused by drugs are the most likely causes. Common causes are listed in Web Box 50-1. Nonhepatic disease is a common cause of ALP elevation.
elevations, resulting from secondary reactive changes (reactive hepatopathies) occurring in the liver. Potential causes include enteritis, pancreatitis, or systemic inflammatory or infectious diseases. Histologic evidence of disease as indicated by a variety of nonspecific reactive changes (e.g., hydropic change and periportal inflammation) often is present. Typically ALP values are threefold to fourfold above normal. Elevations in C-ALP also can result from chronic illness and endogenous glucocorticoid release. Both conditions generally cause only mild ALP elevations.

The highest values of ALP generally are seen with focal or diffuse cholestatic disease, glucocorticoid exposure, chronic hepatitis, and hepatic neoplasia. Concurrent elevations in other liver parameters (alanine aminotransferase [ALT], alanine aspartate aminotransferase [AST], γ-glutamyltransferase [GGT], and total bilirubin) are often helpful in distinguishing a cholestatic disease process from hepatocellular injury. With cholestasis, elevations in ALP, GGT, and bilirubin are expected. Elevations in the leakage enzymes ALT and AST are often present but should be of smaller magnitude when compared with cholestatic enzymes. Liver enzyme elevations primarily caused by ALT or AST are most consistent with diseases causing hepatocellular injury (i.e., chronic hepatitis).

Advanced Evaluation

Symptomatic Patients. For symptomatic patients further evaluation of increased ALP often is required if elevations cannot be explained by nonhepatic diseases, drugs, or endocrine disorders. In these cases primary hepatic or biliary disease is likely, and imaging is essential to further characterize the cause.

Although abdominal radiographs are useful for determining liver size and the presence of other abdominal abnormalities, they lack specificity with regard to hepatobiliary disease. Thoracic radiographs are obtained routinely to screen for metastatic neoplasia or other concurrent disease, especially in older patients, before more invasive diagnostics are directed toward the liver.

Ultrasound is required to characterize more fully abnormalities of the biliary system and hepatic parenchyma. However, one major limitation is the variability in sonographer experience and thus the quality of the imaging procedure. Ultrasound of the hepatic parenchyma may identify masses or nodules, whereas changes in the echogenicity of the liver may suggest the presence of diffuse or focal hepatic disease. The absence of ultrasonographic abnormalities does not rule out liver disease. For example, diffuse hepatic lymphoma can be associated with hyperechoic, hypochoic, and isoechoic hepatic parenchyma on ultrasound. In addition, dogs with benign nodular hyperplasia may have either normal or abnormal ultrasonographic changes. Lesions of the biliary system often consist of dilation of the hepatic and common bile ducts, as well as gallbladder distention, wall thickening, calculi, and sludging. Mass lesions affecting the biliary system also are identified commonly.

Specific lesions in the liver should be sampled with fine-needle aspiration (FNA) or biopsy. Often FNA is performed initially if solitary masses or diffuse hepatic changes are observed because the technique is safe and requires minimal or no sedation. In addition, FNA is often adequate to diagnose neoplasia and is used if diffuse parenchymal disease is suspected. FNA cytology results consistent with neoplasia generally are trusted, but false-positive results are possible. Other findings should be interpreted with caution because FNA and histopathology may have a poor correlation based on the disease involved. Lipidosis and vacuolar hepatopathies are examples of diffuse diseases in which FNA and histology generally correlate well; however, vacuolar hepatopathy may be seen with any number of diseases, including HAC, steroid exposure, and chronic illness (Sepesy et al, 2006). Wedge or laparoscopic liver biopsy is pursued if FNA results are questionable or a histologic diagnosis is required. In addition to biopsy, collection of bile for culture, Gram staining, and cytology are recommended in dogs with evidence of biliary disease.

Preprandial and postprandial bile acids are obtained routinely to further assess liver function if diagnostic evaluation has failed to reveal the underlying cause of
ALP elevation. Abnormal bile acids generally are seen with decreased liver function, acquired or congenital portosystemic shunting, and cholestasis. If results are abnormal, a liver biopsy is warranted because of strong evidence to support the presence of primary liver disease. However, normal bile acids do not rule out significant hepatobiliary disease; thus biopsy also is warranted in patients with or without clinical signs that have persistent or increasing ALP values that are unexplained for longer than 4 to 6 weeks.

**Asymptomatic Patients.** Increased ALP often is found incidentally on preanesthetic or annual blood work. Initial evaluation in these patients should proceed as described previously by ruling out exposure to drugs or supplements and assessing for the possibility of nonhepatic disease, including endocrine, gastrointestinal, and neoplastic disorders. History and physical examination should be repeated to ensure that clinical abnormalities truly are absent.

Options for further evaluation include (1) monitoring ALP over time and (2) pursuing additional diagnostics. For most cases it is appropriate to monitor ALP over 4 to 6 weeks. If progressive or persistent increases occur, further workup is indicated as described previously for symptomatic patients. Alternatively, abdominal ultrasound and bile acid measurements are pursued initially to rule out obvious structural and functional abnormalities of the liver and biliary system because the authors have seen several cases of hepatic neoplasia in asymptomatic patients with ALP as the only clinical abnormality. The decision for additional monitoring or immediate evaluation also may be based somewhat on the degree of ALP elevation. Moderate-to-severe increases are associated more often with hepatobiliary disease or exposure to glucocorticoids and are unlikely to resolve over time.

Common hepatic causes of ALP elevation in asymptomatic patients include neoplasia, benign nodular hyperplasia, chronic hepatitis, idiopathic vacuolar hepatopathy, and breed-related conditions. Benign nodular hyperplasia, idiopathic vacuolar hepatopathy, and breed-related conditions often have ALP increases only, with little or no other liver enzyme involvement (Web Box 50-2). Idiopathic vacuolar hepatopathy is associated with vacuolated hepatocytes containing glycogen. In some of these cases abnormal adrenal production of progesterone or 17-hydroxyprogesterone is thought to be a possible cause (see Chapter 148). Adrenal steroids can be measured in conjunction with adrenocorticotropic hormone stimulation using specific diagnostic laboratories. Some also refer to this condition as atypical HAC because liver biopsy changes are identical to those of a steroid hepatopathy. These patients often do not progress, and therapy is controversial; however, some report that melatonin or typical HAC therapy resolves hepatic changes and the elevations in C-ALP.

### WEB BOX 50-2

**Common Conditions Resulting in Only Serum Alkaline Phosphatase Elevations**

- Hyperadrenocorticism
- Idiopathic vacuolar hepatopathy
- Hepatic neoplasia
- Nodular hyperplasia
- Drug induction
- Breed related

A common finding in Scottish terriers is ALP elevation, often without other concurrent laboratory abnormalities. In a recent study Scottish terriers have been shown to have a higher incidence of increased ALP (Nestor et al, 2006). An additional report describes seven Scottish terriers evaluated for increased ALP with no identifiable cause after thorough imaging, adrenocortical testing, and liver biopsy, suggesting that a benign familial hyperphosphatasemia may be present (Gallagher et al, 2006). A study the authors performed found the ALP to be predominantly C-ALP but failed to reveal an association between elevated endogenous steroid hormone precursors (i.e., 17-hydroxyprogesterone) and elevations in ALP in affected Scottish terriers. In fact, similar abnormalities in 17-hydroxyprogesterone and progesterone were present in terriers with high ALP and those with normal ALP. Currently the underlying cause for elevations in ALP in Scottish terriers is unknown; however, the condition appears to be benign, although concurrent systemic hypertension and renal proteinuria have been observed in many Scottish terriers as well as in dogs with idiopathic vacuolar hepatopathy.

### References and Suggested Reading


