Validation and Verification of Alanine Transaminase as a Biomarker in Canidae and Its Relevance in Veterinary Medicine

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Abstract

In veterinary medicine, Alanine aminotransferase (ALT) is a key biomarker particularly to detect liver injuries in Canidae species. ALT is liver specific enzyme therefore it's concentration in serum make it valuable marker to monitoring hepatic function specifically hepatocellular damage. This chapter delves into the validation and verification of ALT as a reliable biomarker in Canidae and its clinical significance in diagnosing liver disease and other related systemic conditions. Validation involves sensitivity and specificity of ALT across different disease states such as hepatitis, metabolic syndromes, and drug-induced liver injury all prevalent concerns in both wild and domestic Canidae. The chapter also examines the process of verification in term of cross species comparison and variables like age, diet and physical activity that could influence ALT levels to further refine the accuracy and precision of ALT measurements in clinical settings. In prognosis the combined use of ALT biomarker with other biomarkers help to detect most of the liver injuries. The goal of this chapter is to give researcher and veterinarian a thorough understanding of how ALT might improve early identification and therapeutic approaches for liver damage and associated disorders in Canidae. ALT works as a valued biomarker in veterinary medicine through methods that have been defined and verified.

Keywords: Alanine transaminase (ALT), Biomarker validation, Hepatocellular damage, diagnosis veterinary field.

1. Introduction

In veterinary medicine, the field of biomedicine have advanced applications that significantly helping to improve the diagnosis, treatment and prevention of animal diseases. Conventionally the field depends on clinical observations and laboratory tests. However, with biotechnology integration make easy and precise disease diagnosis for animal health and public

safety. Prognosis is important in controlling infectious diseases because delayed detection led to severe health issues in both animals and humans. Therefore, advanced diagnostic tools determined by biotechnological developments are essential for animal reproduction and disease management to effectively reduces health risk (Myers et al., 2017).

Biomarkers are getting more significance in the field of animal health because of their ability to evaluate possible health hazards. They are essential for healthcare tasks like assessing organ toxicity or failure, determine therapy responses and disease-causing factors (Perera et al., 2022). Alanine Aminotransferase (ALT) is commonly identified as a liver-specific enzyme is widely used for detecting liver disease or damage. In healthy dogs, ALT is primarily found within hepatocytes but when these cells experience damage, ALT is released into the bloodstream resulting in increased serum levels. ALT serves as a biomarker or indicator of liver cell injury in both dogs and cats (Stockham & Scott, 2013). An increase in ALT activity levels corresponds to the extent of liver cell damage. However, it does not predict reversible and irreversible liver cell injury, so it lacks prognostic capability. The highest ALT levels generally appear with acute liver cell necrosis and inflammation (Lala et al., 2023).

2. Biomarkers and its Categories

Biomarkers can be categorized in various ways. Traditionally, classification is often based on use. The NIH DWG identified six primary uses: diagnostic, staging, prognostic, predictive, monitoring and as surrogate endpoints. In 2010, an Institute of Medicine (IOM) committee was organized in response to a request from the FDA Center for Food Safety and Applied Nutrition along with the FDA Center for Drug Evaluation and Research (CDER). They outlined ten applications of biomarkers in managing chronic disease and five in drug development (Food et al., 2016). Recently the FDA-NIH BWG proposed seven distinct biomarker types (Myers et al., 2017).

i. Susceptibility/Risk biomarker: Identifies the likelihood of developing a disease or sensitivity to an exposure in individuals without clear symptoms (Stockham & Scott, 2013).

ii. Diagnostic biomarker: A number of factors, including the target pathogen's inherent and native arrangements of antimicrobial vulnerability, the contagion site, drug pharmacokinetics (PK) and pharmacodynamics (PD), host aspects

iii. Monitoring biomarker: Regularly measured to detect disease progression, assess safety or show exposure including exposure to medical treatments (Food et al., 2016).

iv. Predictive biomarker: Helps identify individuals likely to benefit or be adversely affected by a particular intervention or exposure (Myers et al., 2017).

v. Prognostic biomarker: Indicates the probability of a clinical outcome such as disease recurrence or progression (Mobasheri & Cassidy, 2010).

vi. Pharmacodynamics/Response biomarker: Shows that a biological response has occurred following an intervention or exposure (Burke, 2016).

vii. Safety biomarker: Points to the presence or level of toxicity related to an intervention or exposure (Oikonomidis & Milne, 2023).

3. Role of Biomarkers in Veterinary Medicine

a. Disease Diagnosis: Biomarkers are essential in diagnosing various animal diseases. For instance, enzyme immunoassays identify antibodies against specific pathogens facilitating the diagnosis of viral and bacterial infections. Clinical pathology tests are widely employed to diagnose conditions such as chronic kidney failure and diabetes mellitus significantly improving the diagnostic capabilities of veterinary practitioners (Mobasheri & Cassidy, 2010).

b. Monitoring Disease Progression: Numerous traditional biomarkers have been adapted from human medicine for monitoring disease progression in animals. Biomarkers like serum creatinine and proteinuria are crucial for evaluating kidney function over time (Cianciolo et al., 2016).

c. Prognostic Indicators: In veterinary cardiology, biomarkers such as N-terminal pro b-type natriuretic peptide (NT-proBNP) provide valuable prognostic information about heart disease, aiding in treatment strategies and enhancing patient outcomes (Sargent et al., 2015).

d. Cancer Detection and Monitoring: Emerging biomarkers are being utilized in veterinary oncology to detect and monitor cancer disease. Serum thymidine kinase 1 (TK1) for instance is used to detect and monitor lymphomas and other cancers causing agents. Additionally, several biomarkers are being developed for non-invasive cancer screening which help in early detection (Peña et al., 2014).

e. Immunological Assessments: Research is also being conducted on biomarkers to assess the immune system's efficiency and infectious disease susceptibility, especially in animals. Improved health management techniques and less dependency on antimicrobial therapies could come from this investigation (Burke, 2016).

4. Alanine aminotransferase (ALT) Enzyme

The study of enzyme activity in serum, blood, plasma, or other body fluids for diagnostic, prognostic, or monitoring purposes is known as clinical enzymology (Oikonomidis & Milne, 2023). Alanine aminotransferase (ALT) also called serum glutamic pyruvate transaminase (SGPT) facilitates the reversible transfer of amino groups between L-alanine and 2-oxoglutarate, resulting in pyruvate and L-glutamate. To function as an active holoenzyme, ALT depends on pyridoxal 5'-phosphate as a cofactor (Kaneko et al., 2008). ALT is significantly more active in hepatocytes making it a reliable marker for liver cell injury in dogs and cats. While skeletal and cardiac muscles exhibit lower ALT activity compared to the liver extensive muscle injury such as rhabdomyolysis may lead to increased ALT serum levels due to the high overall muscle mass in the body (Lala et al., 2023). ALT half-life in dogs is reported to range widely from 3 to 60 hours though 40–60 hours is often cited as typical (Lawrence & Steiner, 2017). In cats ALT has a shorter estimated half-life of about 3–4 hours (Malakouti et al., 2017).

L-Alanine+ α-Ketoglutarate → Pyruvate+ L-Glutamate

This reaction is crucial for amino acid metabolism reflecting liver function and energy production. ALT facilitates the transfer of an amino group from L-alanine to α-ketoglutarate resulting in the formation of pyruvate and L-glutamate. Pyruvate can then enter further metabolic pathways including its conversion to lactate under anaerobic conditions via lactate dehydrogenase (LDH).

Pyruvate+NADH+H^+→L-Lactate+NAD^+

LDH catalyzes the reduction of pyruvate to L-lactate, simultaneously oxidizing NADH to NAD^+. This reaction plays a significant role in maintaining redox balance within cells particularly during high-energy demands or oxygen-limited conditions (Litwack, 2018).

Function of ALT in dogs: Alanine aminotransferase (ALT) is crucial enzyme in dogs involved in several metabolic functions. It facilitates the conversion of the amino acids alanine and aspartate into pyruvate, which serves as a key energy source for cells. This process is crucial for meeting cellular energy demands and ensuring proper function. In addition, ALT supports the liver's functions such as detoxification maintaining chemical balance in the body and eliminating waste products. Through these roles ALT plays a significant part in sustaining overall health and homeostasis within the body (Lala et al., 2023).

4.1. Organ specificity: ALT is present in the liver, cardiac and skeletal muscles, kidneys and red blood cells of some species. In dogs, cats, rabbits, rats, and primates, ALT is relatively specific to the liver. Although increases in ALT levels may occasionally occur in cases of severe muscle disease in dogs and cats due to enzyme release from muscle tissue. This condition more commonly results in higher AST elevations (Litwack, 2018).

4.2. ALT and Muscle Necrosis: In dogs increased serum ALT activity can also indicate muscle necrosis not just liver damage. Although ALT is typically associated with liver function in cases of significant muscle injury or necrosis. ALT can leak from affected muscle cells resulting in heightened serum levels (Malakouti et al., 2017).
With increased ALT leakage in serum level cause cellular damage leads to an abnormal. The elevation in lactate production which disrupts the balance of NADH and NAD^+ resulting in lactate buildup and potentially leading to lactic acidosis (Ramaiah, 2007). Veterinarians must consider potential muscle-related conditions when encountering elevated ALT levels particularly in dogs displaying symptoms of muscle injury or diseases that affect muscle tissue such as trauma or degenerative muscle disorders (Weingarten & Sande, 2015).

4.3. Elevated ALT level in dogs: Elevated levels of alanine aminotransferase (ALT) are often associated with liver damage, but several non-liver conditions can also cause an increase in ALT. Conditions such as heart failure and intestinal inflammation

can lead to ALT elevations of up to four to five times the severe dental issues may result in elevated ALT levels. Regular exposure to toxins and liver inflammation also contribute to increased ALT levels in dogs (Center, 2007).

4.4. Causes of High ALT Levels in Dogs: Normal ALT levels in dogs range from 6 to 70 IU/L (International Units per Liter). Various factors can cause an increase in ALT levels including the following:

i. CXMD (X-Linked Muscular Dystrophy): CXMD leads to a persistent increase in serum ALT levels. This genetic condition is degenerative and progressive causing breathing difficulties and issues with eating in affected dogs. Tragically, approximately 50% of dogs with CXMD do not survive past 15 months of age and face a significant risk of congestive heart failure (Malakouti et al., 2017).

ii. Hepatocellular Necrosis: Hepatocellular necrosis is another major cause of elevated ALT levels. Any liver injury can lead to a peak in ALT levels typically occurring 1-2 days after the injury. Since ALT has a short half-life levels begin to decrease 2-3 days after the initial rise (Weingarten & Sande, 2015).

iii. Muscle Damage: ALT levels can also rise due to muscle damage. Therefore, when there is a sudden increase in ALT it is crucial to determine whether the cause is liver or muscle-related. Elevated creatine kinase levels can help differentiate between these two conditions. When ALT rises due to muscle injury creatine kinase levels significantly increased (Center, 2007).

iv. Endogenous Causes: Certain extrahepatic diseases that indirectly affect the liver can elevate ALT levels in dogs. For example, canine hyperthyroidism can cause moderate ALT elevation as anticonvulsant therapy and biliary stasis (Lala et al., 2023).

v. Metabolic Cause: Common metabolic conditions associated with increased ALT levels include: Diabetes mellitus, Hyperadrenocorticism, Hyperthyroidism (Malakouti et al., 2017).

vi. Hypoxic Causes: These include hepatic congestion, thrombosis, anemia, seizures and sepsis (McAtee & Lidbury, 2017).

vii. Neoplastic Causes: Cancer-related conditions such as lymphoma, metastatic neoplasia and hepatocellular carcinoma can also lead to increased ALT levels (Center, 2007).

viii. Inflammatory Causes: ALT levels may rise due to liver-related inflammation such as chronic or acute hepatitis, cholangitis, cholangiohepatitis and cholecystitis (McGill, 2016).

ix. Infections: Various infections can cause elevated ALT levels including: Leptospirosis, Histoplasmosis, Feline infectious peritonitis, Schistosomiasis, Ascending bacterial infections (Weingarten & Sande, 2015).

x. Other conditions that may lead to increased ALT include steatosis, benign nodular liver hyperplasia and hemolysis (Center, 2007).

Exogenous Causes: Several external factors can elevate ALT levels in dogs including:

- **Medications:** Certain medications can significantly increase ALT levels. These include: Tetracycline, Carprofen, Phenobarbital, Azathioprine, Antifungal medications, Trimethoprim-sulfadiazine, Lomustine. If dog has been on any of these medications for an extended period be mindful of potential increases in ALT levels.
- **Toxins:** Common toxins that may elevate ALT include cycads, xylitol, zinc, aflatoxins, amanita mushrooms, blue-green algae, carbon tetrachloride and various heavy metals.
- **External trauma:** Any trauma to the liver or muscles can also lead to increased ALT levels (Center, 2007).

4.5. Signs of Elevated ALT Level

a. Common Signs: In early stages it can be challenging to notice signs of elevated ALT levels. Often, dogs do not display symptoms initially. Signs usually become apparent during the acute stage or once 75% or more liver damage has occurred (Tams, 2011).

b. Early Signs of Elevated ALT Levels in Dogs: The early indicators of elevated ALT levels in dogs can be subtle and are often mistaken for other issues. Here are some common early signs:

- **Dullness:** There is a noticeable general lack of vibrancy.
- **Appetite Loss:** It's frequently difficult to pinpoint the exact origin of this symptom.
- **Weakness and Weight Loss:** Inadequate diet may contribute to these symptoms.
- **Unstable Gait:** Weakness can make it harder to stay balanced.
- **Dark Urine:** Tea-colored urine could indicate problems with the liver.
- **Clay-colored or light-colored stool:** This alteration may also indicate liver issues.
- **Skin Itchiness:** A number of internal problems might cause unexplained itching.
- **Disorientation and Decreased Activity:** Dogs may appear confused and less aware of their environment (Tams, 2011).

c. Late Signs

With elevated ALT level late signs appears which cause serious illness. These may include:

- **Jaundice:** Yellowing of the eyes and mucous membranes.
- **Blood in Stool:** This can indicate gastrointestinal bleeding.
- **Seizures:** Neurological symptoms may arise if liver function is significantly compromised.
- **Ascites:** Accumulation of fluid in the abdomen can occur.

It is crucial to observes these signs and symptoms to avoid serious consequences and should consult to the veterinarian (Malakouti et al., 2017).

5. Hepatocellular Injury detector ALT Biomarker

Alanine Aminotransferase (ALT) is the most important indicating biomarker in detection of liver cell damage in dogs and cats. The severity of hepatocellular injury is directly proportional to the elevated level of ALT. The ALT levels increases significantly within 24 to 48 hours highly elevated within five days. ALT level returns to normal baseline if the injuries are not severe within two or three weeks. However, a decrease in ALT may also signal considerable loss of liver cells. Chronic liver diseases often show fluctuating ALT levels which can reflect either recovery or progressive liver degeneration. Certain toxins may not result in elevated ALT levels as they can inhibit the enzyme production in liver (McGill, 2016).

5.1 Role of ALT as a Biomarker with Other Liver Enzymes

- **ALT and ALP:** When evaluating liver diseases ALT is commonly measured alongside Alkaline Phosphatase (ALP). ALT reflects damage specifically to liver cells while ALP is associated with bile duct problems. Analyzing both enzymes helps differentiate between hepatocellular and cholestatic liver diseases (Malakouti et al., 2017).

- **ALT and AST:** ALT is also assessed alongside Aspartate Aminotransferase (AST). Although both enzymes indicate liver damage. ALT is more liver-specific, whereas AST can be elevated due to muscle damage as well. Elevated ALT with AST provides a clearer indication of liver-specific injury (Assawarachan et al., 2023).

- **ALT with Bilirubin:** High bilirubin levels in conjunction with elevated ALT suggest severe liver dysfunction. While ALT indicates early liver cell damage while bilirubin levels reflect more advanced liver disease and the organ impaired ability to eliminate waste products (McAtee & Lidbury, 2017).

6. Biological role of ALT in dogs

- **ALT as a Liver-Specific Enzyme:** Alanine Aminotransferase (ALT) is primarily found in the cytoplasm of liver cells (hepatocytes). When these cells are damaged or destroyed ALT is released into the bloodstream making it a specific marker for liver injury in dogs. Elevated serum ALT levels typically indicate hepatocellular damage (Assawarachan et al., 2023).

- **Phenobarbital-Induced Liver Changes:** In dogs with epilepsy treated with phenobarbital studies have shown increased ALT levels. This may indicate either hepatotoxic effects from the drug or liver enzyme induction where the liver increases its production of enzymes without causing damage. Long-term phenobarbital administration can lead to these changes

necessitating careful monitoring of ALT levels to differentiate between potential liver damage and enzyme induction (McGill, 2016).

- **Correlation between ALT and Liver Histopathology:** Research investigating the relationship between ALT levels and liver histopathology has found that elevated ALT does not always correspond directly with the severity of liver tissue damage. Some dogs exhibited histopathological signs such as hepatic necrosis and inflammation. So far their ALT levels may not reflect these changes proportionally. This indicates that ALT can increase due to cellular stress or enzyme induction before significant liver damage occurs (Menard et al., 2019).

- **Role in Monitoring Liver Health:** Monitoring ALT levels is essential for assessing liver health in dogs particularly those on phenobarbital treatment. Regular measurement of ALT allows veterinarians to evaluate liver function and make necessary adjustments to medication dosages reducing the risk of hepatotoxicity while effectively managing the condition (Gaskill et al., 2005).

7. Validation of ALT as a Biomarker

- **ALT as a Hepatocellular Damage Marker:**

ALT serves as a well-recognized biomarker for hepatocellular injury predominantly released when liver cells (hepatocytes) are damaged (Menard et al., 2019). This quality makes ALT a reliable indicator of liver injury in dogs where elevated serum levels signal either acute or chronic damage to liver cells. This is crucial for assessing the progression of liver disease (Twedt, 2009).

- **Limitations in Fibrosis Staging:**

Despite its role as a sensitive marker for liver cell damage, ALT levels may not be adequate for accurately staging hepatic fibrosis which is the formation of scar tissue in the liver. In chronic liver diseases like fibrosis ALT can remain elevated during the early disease stages. However, ALT level may come to normal or decreases if the number of normal functional hepatocytes decreases as disease progresses. These characteristic limits the ALT usefulness in staging advanced fibrosis (Eulenberg & Lidbury, 2018).

- **Use of ALT with Other Biomarkers:**

It is essential the use of ALT with biomarkers for understanding liver fibrosis progression such as hyaluronic acid and procollagen III peptide. These biomarkers provide important information to of fibrosis changes that may not detect by ALT marker alone. This combined biomarker use validates the results more efficiently (Meurer et al., 2020).

8. Importance of ALT in Liver Function

- **ALT as a Liver-Specific Enzyme:** The primary occurrence of ALT is hepatocytes. During liver damage ALT is released into the bloodstream acting as an indicator of liver injury. For example, infectious hepatitis in dogs. In this condition virus attack on liver cells causing inflammation which leads to serious condition cellular death and hepatocellular injury (Kahilo et al., 2012).
- **Elevated ALT Indicator of Hepatocellular Injury:** Puppies experimentally infected with Canine Adenovirus-1 (CAV-1) which cause liver disease is detected by liver specific enzyme. Tracking ALT levels can aid in detecting early liver injury and evaluating the severity of the disease. (Hu et al., 2001).

- **ALT as a Diagnostic Marker for Liver Health:** In studies on infectious canine hepatitis, ALT measurement is part of a liver function assessment panel. Notably elevated ALT levels correlate with liver inflammation, cell death and compromised liver function, aiding veterinarians in diagnosing ICH and monitoring its progression (Kahilo et al., 2012).

- **Prognostic Use of ALT in Infectious Canine Hepatitis:** In infected puppies veterinarians investigate disease and hepatitis infection by monitoring ALT trends in both terms high and low. The Rapidly increasing ALT level signifies abruptly liver injuries while returning to normal level support damage recovery and injuries healing. This could be only possible if the disease id prognostic at early stages by ALT level (Assawarachan et al., 2023).

- **Liver Necrosis with ALT level:** ALT is liver specific which detect liver damage. In experimental dogs induced rising level of ICH that cause liver necrosis which is estimated by ALT. This sensitivity of ALT biomarker determines necrosis stages and progression because mixed pattern of liver enzyme activity is due to major cause called hepatotoxicity which caused extrahepatic disease or necrosis (Knudsen et al., 2016).

ALT in Differential Diagnosis: ALT's liver specificity makes it a valuable tool for differentiating liver damage from damage in other tissues unlike enzymes such as aspartate aminotransferase (AST) which may rise due to injury in various organs (Ettinger et al., 2016). Consequently, ALT is essential in identifying liver-specific damage among other causes of enzyme elevation For example increased ALT and AST activity result in hepatocellular damage because of leakage od enzyme in bloodstream. But the major difference is that in dogs and feline the ALT is higher in liver cells than AST as AST high concentration is present in variety of tissues of skeletal muscles red blood cells more likely less in liver hepatocytes (Oikonomidis & Milne, 2023).

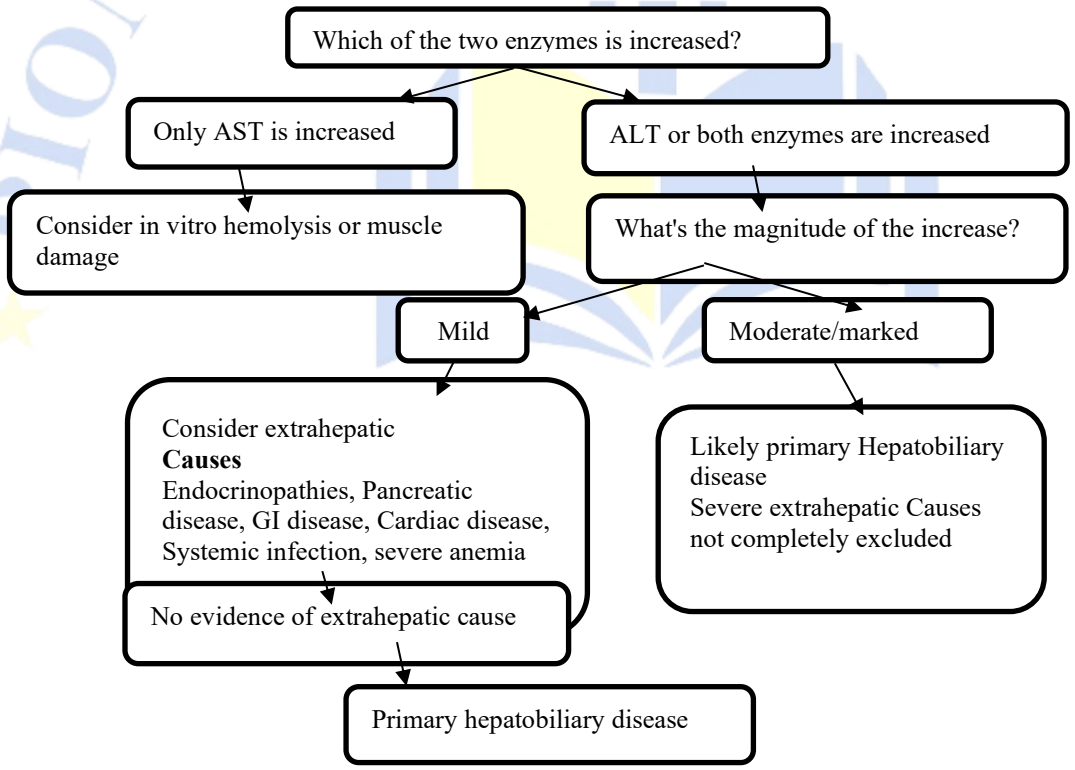

Figure 1: Diagnostic Approach for Elevated Serum ALT and AST in Dogs and Cats: Increases in ALT and AST levels are categorized based on severity

9. Verification Process

Cross-Species Comparison: Abnormal liver biochemistry is frequently observed in both asymptomatic and symptomatic individuals during routine blood screening. Human's studies have shown that around 4% of asymptomatic individuals have elevated serum liver enzymes. Comparatively, a study examining 1,022 blood samples from healthy and unwell dogs and cats found that 39% showed elevated alkaline phosphatase (ALP) while 17% had increased ALT levels (Twedt, 2009).

Findings across Studies: Persistent ALT elevations should be if it exceeds twice then the normal range. Early chronic hepatitis diagnosis and therapy increases survival chances in patients. In dogs' hepatitis often begins between ages 2 to 5 presenting primarily as elevated ALT. Female dogs are affected more frequently and certain breeds are predisposed to hepatitis. In these cases, ALT elevations often accompany bile acid abnormalities and young dogs under one year with high ALT may have portal vascular anomalies which should be exclude by measuring bile acid levels (Han & Kim, 2008).

ALT and AST Interpretation in Veterinary Medicine: Clinical observations support the importance of evaluating ALT and AST levels for liver disease in veterinary practice. Following acute liver injury ALT and AST levels tend to rise markedly. However, due to differences in their half-lives and cellular locations AST levels typically normalize more quickly (within hours to days) than ALT levels (which may take days). This difference aids in assessing the timing and extent of liver damage (Martin & Friedman, 2018).

10. ALT in Diagnostic and Prognostic Use

10.1. Pathophysiology

Liver Disease: Increases in ALT activity can occur in both primary and secondary hepatic diseases due to altered cell membrane permeability or necrosis. However, these increases are not specific to the underlying cause. The highest levels of ALT are typically observed in necrotizing or inflammatory liver conditions. Certain diseases such as hepatocellular carcinoma, hepatic insufficiency, advanced cirrhosis and chronic hepatitis may not show increases in ALT if active hepatocyte injury not occurs. Generally, ALT levels are higher than aspartate aminotransferase (AST) levels in cases of liver disease (Bruyette, 2020).

Bile duct obstruction or cholestasis: In case of bile duct obstruction or cholestasis ALT and AST may increase due to the toxic effects of retained bile salts on hepatocytes. When cholestasis occurs, there is typically a greater increase in cholestatic enzymes such as alkaline phosphate (ALP), gamma-glutamyl transferase (GGT) and bilirubin compared to liver leakage enzymes like ALT and AST. This pattern helps to differentiate cholestasis from other liver conditions and aids in identifying the underlying pathology (Wang et al., 2023). In cases where liver injury leads to cholestasis, leakage enzyme levels will be higher than those of induction enzymes with total bilirubin concentrations potentially normal or only slightly elevated. For instance, chronic hepatitis in dogs often shows higher fold increases in ALT compared to ALP. Here ALT serves as one of the earliest biochemical markers of chronic hepatitis particularly when unexplained increases persist for more than two months with cholestatic enzymes rising later in the disease progression (Webster et al., 2019).

Muscle Disease: In large animals ALT activity can rise with muscle injury but it is not more relevant than AST leading to its exclusion from large animal chemistry panels. For example, in horses ALT activity is highest in heart and muscle tissues compared to the liver making skeletal muscle the primary source of ALT activity due to its mass. In small animals, severe muscle injuries can also lead to elevated ALT. Typically the increases in ALT are less pronounced than in AST with sorbitol dehydrogenase (SDH) levels remaining normal unless there is combined liver injury (Johnson et al., 2019).

10. Limitations of ALT

Sensitivity in Detecting Liver Injury: Although ALT is widely used to detect hepatocellular injury its sensitivity is relatively low reported at around 55%. This means that it successfully identifies liver damage in only half of the cases (Webster et al., 2019).

Comparison to MicroRNA Biomarkers: Recent biomarkers specifically microRNAs such as miR-122 and miR-148a show greater sensitivity for liver injury detection. For instance, miR-122 demonstrated an 84% sensitivity rate significantly than ALT. This indicates that ALT remains valuable but not enough for identifying subclinical or early-stage liver injury (Yi & Fuchs, 2011).

Limitations as a Sole Marker:

ALT levels can be normal in some dogs despite the presence of histological liver damage. This limitation reduces the sensitivity and reliability of sole use of ALT. Therefore, it is essential to use ALT in combination with other more sensitive biomarkers such as miR-122 to improve diagnostic accuracy. Specially for liver related conditions that are combining ALT with AST which provides more reliable and accurate results for disease diagnosis (Wang et al., 2023).

Sensitivity

- **For Acute Hepatitis:**

In biochemical indicator like retrospective experimental study the sensitivity and specificity of three basic liver enzymes ALT, ALP and BA was assessed. The results showed ALT sensitivity about 45% indicating moderate effectiveness in identifying acute liver inflammation ALP and BA 15% respectively.

- **For Chronic Hepatitis:**

The sensitivity improved to 71% making it a more reliable marker for long-term liver inflammation with ALP 35% and BA 13% respectively (Dirksen et al., 2017).

Specificity

The acute and chronic hepatitis specificity of ALT was greater than 90% effective in liver damage diagnosis in dogs. The studies suggest that ALT sensitivity and specificity significantly depend on other conditions such as age and enzyme activity and severity of liver conditions (Dirksen et al., 2017).

- The most significant elevations in ALT levels typically occur with hepatocellular necrosis and inflammation. In these cases, a gradual decline in ALT activity over time can indicate recovery. Specifically, in acute liver disease, a decrease of 50% or more in serum ALT levels over the course of a few days is often seen as a positive prognostic indicator (Yi & Fuchs, 2011).

- In acute hepatocellular necrosis, serum ALT levels typically rise between 24 to 48 hours, often exceeding 100 times the normal value. These levels generally peak within the first 5 days after the injury. If the cause of the liver damage is recovered ALT activity tends to gradually return to normal over a period of 2 to 3 weeks (Yi & Fuchs, 2011).

- ALT is highly sensitive for liver injuries diagnosis for example in Infectious hepatitis in dogs ALT rise up to 30-fold than normal level within 4 days. In some cases, it increases to 10-fold lead to fluctuation in ALT level depending on toxin exposure and injury (Center, 2007).

- Beside its high sensitivity in detecting liver injuries one of challenge of ALT biomarker is with its specificity which is least reliable in clinical signification or in identifying specific histopathological issues. Inappropriately the high ALT sensitivity is not correlated to high specificity in identification of hepatic dysfunction (Dirksen et al., 2017).

- Several other conditions and medications that are associated to liver changes causes elevated ALT activity more than tenfold increase. These may include pancreatic diseases, hypoxia and bile or gastric juice related conditions. Certain antibiotics for example corticosteroids and phenobarbital effectively increase serum ALT activity lead to extrahepatic conditions (Bruyette, 2020).

12. Liver Disease Management in Canine

a. Antioxidants and Nutraceuticals:

Oral supplements of S-adenosylmethionine (SAMe) increase level of glutathione in livers of dogs and cats. This balance the antioxidant and ROS level in hepatocytes and red blood cells. Alternatively N-acetylcysteine (NAC) also act as glutathione precursor which support detoxification in case of cholestasis and inflammation (Dirksen et al., 2017).

b. Anti-inflammatory Therapy:

For anti-inflammatory anti immune-modulatory and anti-fibrotic functions corticosteroids and glucocorticoids are prescribed. However their use and dose should be closely monitored because of their side effects such as increased protein catabolism gastrointestinal tract infection, ulceration due to elevated ALP levels increase infection risks in dogs (Davies, 2016).

c. Ursodeoxycholic Acid (Ursodiol):

Ursodeoxycholic acid is used against bile acid which dissolve cholesterol or gall stones. This reduces the bile acid buildup in liver by improving bile flow normally in liver. In veterinary medicine it is use for chronic hepatitis and hepatobillary toxicity (Bruyette, 2020).

d. Colchicine for Hepatic Fibrosis

Colchicine is sometimes used to manage liver fibrosis by slowing collagen buildup and promoting its breakdown. This approach is applied cautiously due to potential gastrointestinal side effects and variable efficacy (Burke, 2016).

e. Dietary Adjustments

In cases involving hepatic encephalopathy, a moderate-protein, high-quality diet is beneficial as it reduces ammonia levels produced liver-metabolized byproduct of protein digestion that may contribute to neurotoxicity when the liver is compromised (Tams, 2011).

13. Conclusion

Alanine Transaminase (ALT) is a crucial biomarker in diagnosing liver health in Canidae, particularly useful for early detection of liver injuries leading to timely interventions and improved outcomes. Elevated ALT generally signals liver disease, yet it must be considered alongside clinical signs and other biomarkers for accurate diagnosis. Research is ongoing to enhance ALT's diagnostic reliability making it essential in canine veterinary medicine.

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