A Laboratory Diagnostic Approach to Hepatobiliary Disease in Small Animals

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KEYWORDS

• Hepatic disease • Enzymes • Bile acids • Bilirubin • Proteins

KEY POINTS

- Liver enzymes can be classified as leakage or induction enzymes; although they are sensitive indicators for detection of disease and/or cholestasis, they often are not specific for a primary cause.
- Increased serum enzyme activities can occur in clinically normal animals, and in animals with hepatic and nonhepatic disease.
- Serum bile acids and ammonia can be measured to evaluate hepatic function.
- Detection of other biochemical abnormalities, such as hypoproteinemia, hypoglycemia, or hypocholesterolemia, may be useful. However, these analytes are often not sensitive indicators of liver disease.

TESTING AND REFERENCE INTERVALS

Understanding the limitations of laboratory diagnostic tests for hepatobiliary disease is important in avoiding misinterpretation of results. Most of these tests are quantitative assays performed on serum or plasma samples and measured on a continuous scale. Clinical interpretation of tests results is guided by reference intervals (RI), but there is inherent overlap in results between clinically healthy and diseased animals. Animals with clinically significant disease can have "normal" test results, and clinically healthy animals can have "abnormal" results.

Determination of RI varies based on the number of test subjects (minimum of 40 based on The American Society for Veterinary Clinical Pathology guidelines) and distribution of data (Gaussian vs nonparametric).[1] Intervals are often established based

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on results from the central 95% of the reference group. Therefore, values from 5% of the test population of clinically healthy animals will fall outside the established interval. In effect, 2.5% of the reference population will have values above or below the RI. RI for a given analyte may vary between laboratories, and comparison of results obtained from different instruments or using different methodology must be done with caution. Adherence to quality-control guidelines and instrument maintenance with proper sample collection and handling are paramount for consistently obtaining accurate results and minimizing variation attributable to instrument or operator error.

OVERVIEW OF HEPATIC ENZYMES

- Enzymes are categorized into leakage enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST]) indicating hepatocellular injury, and induction enzymes (alkaline phosphatase [ALP], γ-glutamyltransferase [GGT]) associated with increased synthesis.
- The severity of serum-activity elevation for leakage enzymes depends on the cellular concentration and the subcellular location. Elevation of serum activity from enzymes found within the cytosol and mitochondria suggests a greater degree of cell injury than elevation of activity from enzymes found within the cytosol alone.
- Induction enzymes are typically associated with the cell membrane, and severity of the serum-activity elevation depends on the capacity for enzyme production.
- The severity of increase of serum enzyme activity may be interpreted as a fold elevation above the upper limit of the RI. A 2-fold to 3-fold elevation is often considered of mild severity. A 4-fold to 5-fold elevation and greater than 10-fold elevation may be considered of moderate and marked severity, respectively.[2]
- The finding of elevated liver enzyme activity is commonly encountered during routine biochemical testing. Detection of a single elevated liver enzyme, particularly of mild severity, may be nonspecific and insufficient for the diagnosis of hepatobiliary disease. In a study of biochemical abnormalities detected in a large population of healthy and diseased canines of various ages, elevations of serum activity for both leakage enzymes (ALT, AST) and induction enzymes (ALP, GGT) was observed in approximately 17%, 11%, 39%, and 19% of the cases, respectively.[3]
- The diagnostic utility of serum enzyme measurement for detection of hepatobiliary disease is enhanced by evaluation of both leakage and induction enzymes, as well as correlation with additional diagnostics such as hepatic function tests or histopathology.
- The duration of increase for leakage and induction enzymes depends on the rate of clearance or half-life, and on the nature and severity of the inciting cause.

Alanine Aminotransferase

ALT is found in high concentrations in the cytoplasm of canine and feline hepatocytes, and is a useful marker of hepatocellular injury in dogs and cats. This enzyme is a catalyst in a reaction resulting in deamination of alanine with production of pyruvate for entry into the Krebs cycle or gluconeogenesis pathway.[4] The enzyme is also found in cardiac and skeletal muscle cells, renal epithelial cells, and red blood cells (RBCs). The primary clinical utility of this enzyme is detection of hepatocellular injury, although enzyme distribution in other tissues can present a diagnostic