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AST/ALT ratio: A new approach over old biochemistry tools[1]

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ABSTRACT.- Machado A.L.F., Bastos L.M., Santos L.B., Sousa M.F., Couto M.P.V., Küster P.H.P., Oliveira L.E.D., Carvalho M.P.N. & Paes Leme F.O. 2024. **AST/ALT ratio: A new approach over old biochemistry tools.** *Pesquisa Veterinária Brasileira 44:e07470, 2024.* Departamento de Clínica e Cirurgia Veterinária, Faculdade de Medicina Veterinária, Universidade Federal de Minas Gerais, Campus Pampulha, Av. Pres. Antônio Carlos 6627, São Luiz, Belo Horizonte, MG 31270-901, Brazil. E-mail: biapatovet@gmail.com

This study aimed to evaluate the aspartate transaminase/alanine transaminase (AST/ALT) ratio in healthy dogs and dogs with hepatic and extrahepatic diseases. Twelve different groups of animals were considered in the study: Control, patients with Acute hepatopathy, and Chronic hepatopathy and patients with extrahepatic diseases such as Pyometra, Fractures/trauma, Intoxication/poisoning, Leishmaniosis, Hemoparasitosis, Oncologic, Gastrointestinal, Skin problems and Nephropathy. A retrospective study was made with 509 exams. Hematological and serum biochemical results correlated to the ratio at time zero (M0) and 48 hours (M48) after the first care, allowing for the prediction of the outcome. Animals with Acute hepatopathy showed AST/ALT ratios 84% above the upper limit of the ranges obtained from Control animals. Animals with Chronic hepatopathy showed higher averages than acute. Animals from Pyometra, Fractures/trauma, and Intoxication/poisoning groups showed higher averages of the AST/ALT ratio (2.67, 2.54, 2.21) than those from other groups. The correlation between the AST/ALT ratio in serial assessments showed that when animals double the value of the ratio in 48 hours, they tend to have a 2.5 greater probability of dying.

INDEX TERMS: Biomarker, AST/ALT ratio, extrahepatic disease, hepatopathy, transaminases.

RESUMO.- [**Razão AST/ALT: uma nova abordagem para analitos bioquímicos antigos.**] Este estudo objetivou avaliar a razão AST/ALT em cães saudáveis e cães apresentando doenças hepáticas e extra-hepáticas. Doze grupos diferentes de animais foram considerados no estudo, como um grupo Controle, pacientes com Hepatopatia aguda e Hepatopatia crônica, e grupos com doenças extra-hepáticas como Piometra, Fraturas/trauma, Intoxicação/envenenamento, Leishmaniose, Hemoparasitose, Oncológico, Gastrointestinal, Afecções de pele e Neuropatia. Um estudo retrospectivo foi realizado utilizando 509 exames. Exames hematológicos e bioquímicos foram correlacionados com a razão no tempo zero (M0) e 48 horas (M48) depois do primeiro atendimento, permitindo predizer a consequência. Animais com Hepatopatias agudas apresentaram razão AST/ALT 84% acima do limite inferior das médias observadas nos animais do grupo Controle. Animais com Hepatopatias crônicas apresentaram médias maiores do que Hepatopatias agudas. Animais presentes nos grupos Piometra, Fraturas/trauma e Intoxicação/envenenamento apresentaram as maiores médias da razão AST/ALT (2,67; 2,54; 2,21) quando comparado aos outros grupos. A correlação entre a razão AST/ALT em avaliações seriadas mostraram que, quando o valor da razão dobra em 48 horas, aumenta em 2,5 vezes a probabilidade de morte.

TERMOS DE INDEXAÇÃO: Biomarcadores, razão AST/ALT, doenças extra-hepáticas, hepatopatias, transaminases.

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INTRODUCTION

Aspartate aminotransferase (AST) catalyzes the conversion of aspartate to oxaloacetate in the Krebs cycle. It can be found inside the mitochondria in hepatocytes but can also be observed in its cytoplasm, in addition to erythrocytes, muscle cells, and renal and pancreatic parenchyma; this deserves special attention in cases of differential diagnosis (Silva 2015). Slight-to-moderate increases in its activity can be observed in samples that have hemolysis *in vitro* or even when clot removal from serum samples is delayed (Stockham & Scott 2011). Its half-life is around 12 hours in dogs; as expected in alanine aminotransferase (ALT) activity, its increase is described in acute liver diseases and active conditions of hepatic necrosis and lipidosis. In chronic processes, this enzyme tends to present activity within the reference range (for dogs, 0-66U/L) (Kaneko et al. 2008, Silva 2015). The main serum AST isoenzyme found, as compared to the size of the tissue, is that from skeletal striated muscle origin. Therefore, it is recommended to be associated with its measure of creatinine phosphokinase (CK) activity, preferably the isoenzyme of muscle origin, NM, to predict the origin of the increase in its activity (Cerqueira 2017).

ALT is a cytoplasmic enzyme that catalyzes the deamination reaction of alanine to pyruvate and is related to gluconeogenesis in the Krebs cycle. In dogs, its half-life ranges from two to three days (48-72 hours) and is the most common enzyme in the hepatocyte cytoplasm; however, it is not considered a liver-specific enzyme since 5 to 25% of its activity is also found in skeletal and cardiac muscles. Although the total muscle mass is higher than the hepatic mass, the activity of this enzyme is more strongly correlated with hepatic biochemical profiles than with muscle mass (Allison 2015, Stockham & Scott 2011). Generally, an increase in ALT activity is observed in the acute process caused by hypoxia, inflammation, neoplasia, drugs (mainly corticosteroids and anticonvulsants), and toxemic processes, and in the liver recovery phase when hepatocytes are regenerating. In chronic processes, such as the terminal phase of liver disease, this enzyme is often found within normal limits or below the reference interval for the species (Allison 2015).

Hepatopathy has largely been used to describe liver alterations that have an acute or chronic course and thus must be investigated. Those changes related to hepatocytes and biliary cells may have different origins, such as congenital or hereditary diseases (Watson 2017), hypoxia, intoxications, metabolic diseases, inflammation, neoplasia, obstructions, or even come from the hematological vial (Praharaj & Anand 2021). It is important to emphasize that, even though this disturbance's origin is often not necessarily hepatic, this organ and its function can be affected indirectly (Allison 2015).

Owing to its high functional reserve capacity, storage, and regenerative activity, up to 70% parenchymal involvement is necessary for laboratory examinations to show functional impairment. Acute and chronic liver failure are liver diseases that have distinct characteristics (Favier 2009). In medicine, it is hard to establish a specific definition of acute liver failure since it overlaps the lesion and can occur suddenly or progressively, related to successive cellular damage, however potentially reversible (Favier 2009, Pedroso & Maestri 2019).

Fernando De Ritis (De Ritis et al. 2006) evaluated 121 human cases of increased transaminase activity (AST and ALT) and observed that the increase in both enzymes, with inversion in their proportion, could be used to guide the diagnosis of acute viral hepatitis, calling the ratio between the two transaminases as the "De Ritis ratio". This ratio provides a numerical variable that can provide information about the course and aggressiveness of liver disease, as predicted from the relatively short half-life of AST compared to ALT and the extent to which cell injury occurs (Botros & Sikaris 2013). A clinical trial conducted by Williams & Hoofnagle (1988) showed that in cases of human chronic hepatitis associated with cirrhosis, the AST/ALT ratio was significantly higher. The authors also stated that values up to 1.0 in cases of chronic hepatitis B suggest that cirrhosis is present or in development.

The main studies on the AST/ALT ratio in medicine correlate its prognostic value not only to liver diseases (Giannini et al. 2002), including primary liver carcinoma (Yang et al. 2018), but also to extrahepatic alterations, such as stroke (Gao et al. 2017), metastatic and non-metastatic renal cell carcinoma (Bezan et al. 2015, Canat et al. 2018, Kang et al. 2018), transitional cell carcinoma of the bladder (Lee et al. 2017, Yuk et al. 2019), pancreatic cancer (Riedl et al. 2020), and nasopharyngeal carcinoma (Wu et al. 2019b, Knittelfelder et al. 2020), among other neoplasms (Wu et al. 2019a) and in hospitalized patients with suspected COVID-19 (Zinellu et al. 2021).

Although the AST/ALT ratio has proven to be a valuable tool in medicine, only a few scientific articles have investigated its use in animals. A study published in 1987 was the first involving the AST/ALT ratio in a veterinary clinical trial and characterized its value in healthy female dogs, not spayed, and demonstrated a significant increase in dogs with pyometra (Schepper et al. 1987) and naturally infected with *Babesia canis* (Zygner et al. 2012). Although the authors discussed systemic involvement, they did not post the prognosis of the ratio (Zygner et al. 2012).

To better understand the AST/ALT ratio (De Ritis) and its utility in veterinary medicine, this study aimed to analyze hematological and serum biochemical results from dogs with and without liver diseases, changes in enzymes, the average AST/ALT ratio in hepatic and extrahepatic diseases, and its correlation with the outcome (alive/death) of hospitalized dogs.

MATERIALS AND METHODS

Ethical approval. Since this is a retrospective study and all the data were obtained from case profiles at the Veterinary Hospital, this study did not perform any animal experiments. Submitting to the Ethics Committee on Animal Use (CEUA) was unnecessary.

The analytical study involved obtaining data from 302 dogs treated at the Veterinary Hospital of the "Universidade Federal de Minas Gerais" (UFMG) and analyzing 509 results. The biochemical profile included urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), glucose, total protein (TP), albumin (ALB), and globulin. Cases without a conclusion of clinical suspicion, with a lack of hematological results, or in a previously defined biochemical profile, as well as cases of intensely hemolyzed or lipemic serum samples, were excluded. The average age of the dogs in this study was eight years, sampled from Dalmatians, Dobermans, Pinschers, English Springer Spaniels, American Cocker Spaniels, and mixed-breed animals.

The biochemical profile was semi-automatically processed (COBAS Mira plus® – Roche) in which the concentrations of urea, creatinine, glucose, total protein (TP), and albumin (ALB), as well as the activities of aspartate (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma GT using commercial reagents from Biotécnica® (Varginha/MG) were determined. For all analyses, the protocols presented in the package inserts of the commercial kits were followed according to the standard routine laboratory protocol (certified by ANVISA).

Statistical analyses were applied to the AST/ALT ratio variable described through mean values and standard deviations, separating the animals into Control and Hepatopathies at first and further subdividing them into Acute hepatopathy and Chronic hepatopathy. The non-parametric Kruskal-Wallis test was applied to test the differences between groups (Control × Hepatopathy × Acute hepatopathy × Chronic hepatopathy). Animals were separated according to the clinical diagnosis (Acute or Chronic hepatopathy), added to the biochemical profile, and subjected to ultrasound, following the criteria proposed by Favier (2009).

Hematological values were obtained using an automatic analyzer (Icounter Vet Diagno), and a blood smear was used for morphological characterization. A table of pairwise correlations between the AST/ALT ratio and all numerical variables (multivariate analysis) was constructed after compiling the data, which analyzed all variables together, considering the correlation between them. Dunn's multiple comparison tests (*post-doc*) were also applied to all variables in all groups evaluated.

Non-parametric Kruskal-Wallis tests were performed using the same analytes to analyze the AST/ALT ratio in extrahepatic diseases. The groups were divided into control and patients who were classified as sick according to the anamnesis, diagnosis listed in the clinical report, or based on the results of laboratory tests: cystitis/urolithiasis, fracture/trauma, gastrointestinal diseases, hemoparasitosis, hepatopathy, intoxication/poisoning, leishmaniasis, drug action, nephropathy, cancer, pyometra, and dermatological conditions. subgroups were formed based on the analysis of the following larger groups: acute and chronic hepatopathy, chronic kidney disease and acute kidney injury, enteritis and gastritis, leishmaniasis with acute kidney injury, cancer patients at the time of diagnosis (untreated) and treatment, and simple and systemic dermatological conditions.

The average AST/ALT ratio of the groups that had patients with at least two laboratory evaluations (0h and 48h) was calculated to analyze the effect of treatment by time and prognostic evaluation and to assess the evolution and stabilization of the case based on the average AST/ALT ratio.

A logistic regression model was adjusted to assess the effect of the AST/ALT ratio on the chance of death, which is suitable for dichotomous data (live/dead), and the odds ratio of death was calculated together with the confidence interval. All statistical analyses were performed using R software version 3.6.1 (R Core Team 2019).

RESULTS

Considering the 302 cases in this study, 83 (27.5%) were allocated to the Control group. In the Hepatopathy group, 76 cases were included and subdivided into Acute hepatopathy (n=61) or Chronic hepatopathy (n=15) groups. The means and standard deviations of the activities of hepatocellular injury (AST and ALT) and induction/cholestasis enzymes (ALP and GGT) were analyzed and found to be associated with urea, creatinine, glucose, total protein, and albumin levels (Table 1). In the Control group, all enzymes showed activity within the reference range for dogs. In the Hepatopathy group, the activities of induction and lesion enzymes, as well as urea and creatinine concentrations, showed elevated serum means and standard deviations.

From the analyzed data, the Hepatopathy group subgroup Chronic hepatopathy showed more evident anemia (hematocrit 30±8) than Acute hepatopathy (hematocrit 33±8; $p<0.05$). In the leucogram, the Hepatopathy group showed leukocytosis (19,432±8,815), higher in the Chronic hepatopathy (24,428±15,600) group than in the Acute hepatopathy (18,077±6,771; $p<0.05$).

Correlations between the principal variables analyzed and the AST/ALT ratio were established. GGT, ALP, ALT, and albumin levels were inversely correlated with the AST/ALT ratio -0.54, -0.49, -0.47 and -0.46 ($p<0.01$), respectively.

For each group and subgroup analyzed in this study, multivariate analysis was performed to obtain the specific correlations of each group profile. In the profile of the Control group animals, the predicted variables that showed a better response were glucose, MCHC, albumin, and hematocrit, which were normally associated with higher results and lower values of total leukocytes and band neutrophils.

When analyzing the Hepatopathy and Acute hepatopathy groups, the general population was represented by a spaced and multivariate profile. Most patients in the population represent an acute phase of the disease. It is possible to say

Table 1. Means and standard deviations of serum biochemical analysis from dogs in Control and Hepatopathy groups subdivided into Acute and Chronic hepatopathy

	Reference range*	Groups			
		Control (n=83)	Hepatopathy (n=76)	Acute hepatopathy (n=61)	Chronic hepatopathy (n=15)
ALT	0 – 102 UI/L	47.06 ± 26.34^{a}	183.1 ± 183.7^{b}	223 ± 188.1^{b}	36.23 ± 22.41^{a}
AST	0 – 66 UI/L	29.12 ± 7.84^{a}	213.4 ± 431.6^{b}	257.3 ± 477.8^{b}	51.64 ± 27.44^{b}
ALP	0 – 156 UI/L	68.44 ± 46.31^{a}	708.3 ± 1145^{b}	807.7 ± 1269^{b}	341.6 ± 254.2^{b}
GGT	0 – 10 UI/L	6.36 ± 8.71^{a}	22.1 ± 49.6^{b}	25.9 ± 55.31^{b}	8.43 ± 7.69^{ab}
Urea	21 – 60 mg/dL	42.3 ± 27.13^{a}	103.8 ± 101.5^{b}	122.5 ± 106.1^{b}	34.98 ± 30.45^{a}
Creatinine	0.5 – 1.5 mg/dL	0.94 ± 0.44^{a}	2.24 ± 2.63^{b}	2.65 ± 2.83^{b}	5.28 ± 18.06^{a}
Glucose	65 – 118 mg/dL	100.3 ± 28.9^{a}	93.43 ± 41.02^{a}	96.58 ± 42.84^{a}	81.81 ± 31.95^{a}
Protein	5.4 – 7.1g/dL	6.1 ± 0.83^{a}	5.28 ± 1.4^{b}	5.43 ± 1.26^{b}	4.71 ± 1.8^{b}
Albumin	2.6 – 3.3 g/dL	2.96 ± 0.45^{a}	2.16 ± 0.69^{bc}	2.29 ± 0.66^{b}	1.62 ± 0.5^{c}

* Reference range (Kaneko et al. 2008); ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyltransferase, n = number of exams; ^{a,b,c} Different letters in the same line show statistical difference in Kruskal-Wallis test ($p<0.05$).

that there exists a positive correlation between AST/ALT ratio, globulin, and creatinine and an inverse correlation with albumin and platelets.

The Chronic hepatopathy subgroup presented an escape profile and elevated values of total leukocytes and band neutrophils, initially giving them the characteristics of predictive variables for this group. In 15 cases that were characterized as belonging to the Chronic hepatopathy subgroup, eight dogs presented with ultrasound alteration with mineralization and possible liver abscess, five had bacterial endometritis, and two were diagnosed with toxic plant poisoning. The importance of analyzing each case is to observe the diagnostic pattern in the group since dogs with primary affection had more intense and irreversible liver damage.

The second group to present a higher increase in AST/ALT ratio was Intoxication/poisoning (2.54±2.16). The trauma/fracture group had a higher AST/ALT ratio (2.21±1.75). The Leishmaniosis group also presented a higher average ratio value (1.67±1.26), associated with an increase in ALT (74.06UI/L) and AST (105.92UI/L) activities. AST/ALT ratio in the Oncologic group presented an average of 1.07±0.76.

A multivariate analysis of patients with acute kidney injury was performed based on all analyzed variables to restrict the evaluation of the Nephropathy group to a specific profile. Although its population has been spaced out, a good finding was in the quadrant represented by higher AST/ALT ratio values, where globulin and creatinine were also present (Fig.1).

Five hundred nine biochemical examinations were included to evaluate the prognostic value of the AST/ALT ratio as a death response independent of the group. From this total, 37 exams represented the death group, with an average and standard deviation of 1.73±0.81 to AST/ALT ratio. As the ratio doubled increased 2.5 times the chance of death (Fig.2).

DISCUSSION

An increase in enzyme activity has been recognized and correlated with lesions in hepatocytes. After the lesion, the hepatocytes present changes in the membrane permeability, leading to cytoplasmic and mitochondrial enzymatic extravasation, increasing their serum activities (Allison 2015). Probably because it represents most of the population in the Hepatopathy group, the Acute hepatopathy subgroup presented the same analysis profile as its group of origin.

In the Chronic hepatopathy subgroup, AST, ALT, and GGT showed quite different serum activities from the Acute hepatopathy group. After constant lesions and hepatic insults, the consequent evolution is the substitution of parenchyma for fibrous conjunctive tissue. The amount of AST and ALT in the hepatocytes to be released decreased, as Allison (2015) presented. Although liver biopsy *ante mortem* is indicated in veterinary cases with suspected cirrhosis, it is not routine (Eulenberg & Lidbury 2018).; in these cases, imaging examinations are important for the analysis and follow-up of the liver parenchyma (Silva 2015, Silva et al. 2020). In the patients in this study, alterations considered as liver fibrosis observed on ultrasound were important variables for relocation to the Chronic hepatopathy subgroup. Molecules requiring liver synthesis reduce their serum concentrations below the reference range, as seen in urea, total protein, and albumin. Although glucose is metabolized and stored in the liver, the average value remains within the reference range. According to some authors, hypoglycemia, even in chronic hepatopathies, is rare, although it is associated with the worst prognosis when present, as described by Allison (2015) and Silva (2015).

Considering anemia found in the Chronic hepatopathy subgroup, it can be correlated with the suppression or failure of erythropoiesis due to inflammatory liver disease, reduction in the synthesis and metabolism of hormones and vitamins, and storage of important minerals, all necessary for erythrocyte production. Evaluation of hemoglobin concentration showed a significant decrease (Control group 15.2±2.28, Chronic hepatopathy subgroup 9.52±2.89; $p<0.05$), resulting in hypochromic anemia, which can be related to iron deficiency due to inadequate reuse of this mineral, which can also be related to inflammatory disease (Stockham & Scott 2011). The observed leukocytosis (24,428±15,600) presented a neutrophilic nuclear shift to the left (band cells 1,602±2,186), corroborating the relationship between hepatopathies and inflammatory processes described by Bexfield & Watson (2006).

The AST/ALT ratios obtained from the analyzed groups are shown in Table 2. In the Control group, the mean found was 0.76, and the standard deviation was 0.39 (min. 0.15, max. 1.83). Previous studies obtained mean values in healthy

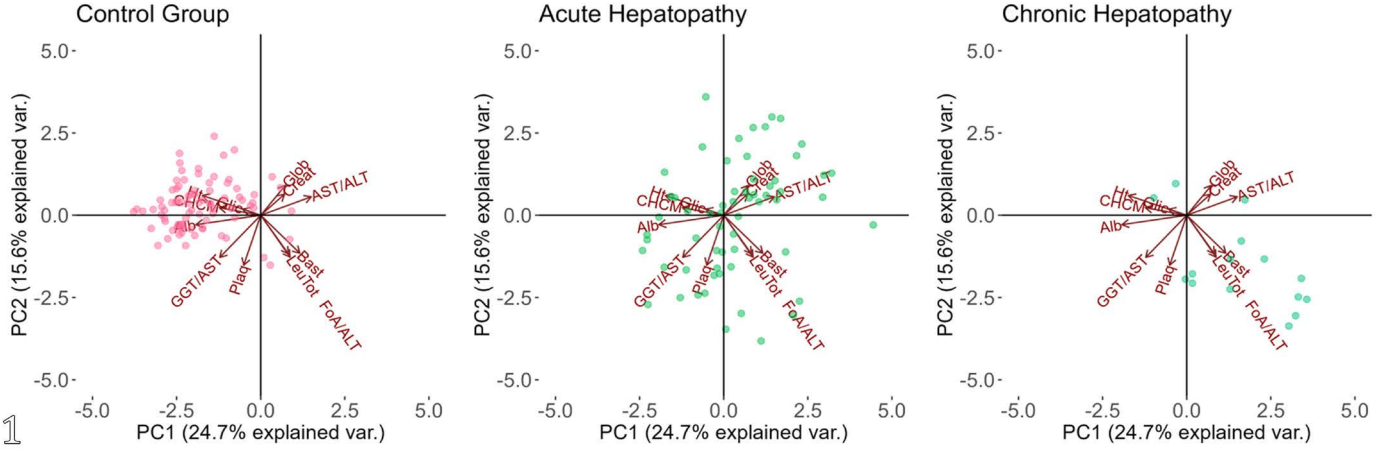

Fig.1. Multivariate analysis in the Control group and Acute and Chronic hepatopathy subgroups

female dogs of 0.6±0.2 (Schepper et al. 1987), like what was found in this study for the considered healthy dogs.

Although possible, it is unlikely that age, sex, and breed discretely influence this ratio. Female dogs represented a proportion of 1.5:1 of males of the same breed. Analyzing the mean and standard deviation in the Hepatopathy group, the AST/ALT ratio (1.37±1.43) showed superior values than the Control group (0.75±0.39), although without statistical difference. Another relevant observation relates to the amplitude of the results observed in this group. As shown in Figure 1, there was great heterogeneity, ranging from animals with values like those of the Control group to animals with ratios twice as high. The mean and standard deviation obtained in the Acute hepatopathy subgroup 1.29±1.54 (min 0.09, max 7.9) corroborates this statement, which also did not show a statistical difference. Notably, the smaller ratio observed in this group was 0.09, which is six times (84%) greater than the higher AST/ALT ratio shown in the Control group animals, demonstrating the severity of the individual changes. In the Chronic hepatopathy subgroup, the mean was higher (1.71), and the standard deviation was 0.70 (min 0.67, max 3.08), with statistically different values when compared to the Control group and 62% higher (Fig.1).

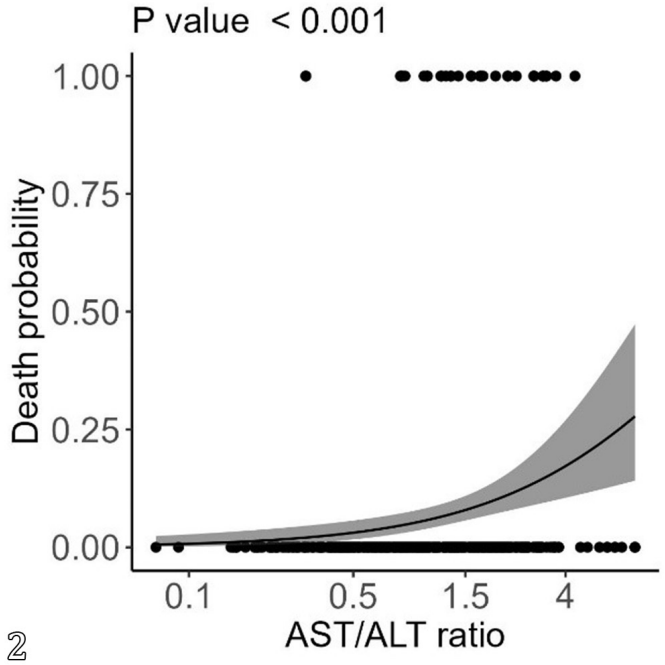

Fig.2. Correlation between aspartate transaminase/alanine transaminase (AST/ALT) ratio with death probability. p<0.001.

Table 2. Means and standard deviations of AST/ALT ratio from dogs in Control and Hepatopathy groups subdivided into Acute and Chronic hepatopahty

Groups	AST/ALT ratio	Standard deviation
Control (n=83)	0.76[a]	0.39
Hepatopathy (n=76)	1.37[ab]	1.43
Acute hepatopathy (n=61)	1.29[ab]	1.54
Chronic hepatopathy (n=15)	1.71[b]	0.79

AST = aspartate aminotransferase, ALT = alanine aminotransferase, n = number of exams; [a,b] Different letters represent statistical differences in the Kruskal-Wallis test (p<0.001).

More than 60 years ago, De Ritis reported that AST/ALT ratio aids in the diagnosis of acute viral hepatitis. Over time, this ratio has been useful beyond acute cases in humans. Gitlin (1982) demonstrated intervals from 0.31 to 0.63 in patients with acute viral hepatitis that survived, against intervals of 1.20 to 2.26 in non-survivals, almost 25% higher. Both Williams & Hoofnagle (1988) and Karim et al. (2015), studying chronic hepatitis, showed a positive correlation between ratios greater than 1.0 and human cirrhosis. In addition, many authors have proven that this ratio is useful for establishing prognosis in several neoplasms (Yuk et al. 2019, Knittelfelder et al. 2020, Riedl et al. 2020, Wu et al. 2019b, vascular diseases (Gao et al. 2017), and systemic infections (Zinellu et al. 2021). In all studies, there was a correlation between an increase in the ratio and an unfavorable prognosis and death. Considering the intervals, it is not possible to use an AST/ALT ratio higher than 1.0 in veterinary medicine as a cutoff for cirrhosis diagnosis since both the Control and Acute hepatopathy groups presented values above 1.0. However, the observed statistical difference may allow us to conclude that patients with chronic hepatitis gradually increase the AST/ALT ratio as the disease progresses, which should be monitored.

In veterinary studies, data obtained from female dogs with pyometra showed increased AST activity and reduced ALT, consequently raising the AST/ALT ratio to values close to 2.5 (Schepper et al. 1987). Another study in dogs affected by *Babesia canis* compared the effects of anemia and azotemia on the AST/ALT ratio in acute and chronic liver lesion cases and considered that azotemia has a positive correlation with the AST/ALT ratio increase, suggesting that renal lesions lead to an increase in AST activity in dogs (Zygner et al. 2012). Unfortunately, the group did not clarify the mechanism and how it could be related to the AST/ALT ratio; however, an increase in AST serum activity may occur due to the addition of an isoenzyme of renal origin or due to hepatorenal syndrome.

Correlations between the principal variables analyzed and the AST/ALT ratio were performed due to the lack of information on veterinary medicine in response to which analytes correlate significantly with the ratio studied. A negative relationship to albumin values found in this study has already been documented, but without a statistical difference, by Karim et al. (2015).

Albumin is an important biochemical analyte that is used to evaluate liver function. Since its synthesis depends exclusively on hepatocyte integrity, patients with chronic liver disease progressing to cirrhosis have reduced serum concentrations of this protein. Surprisingly, there was a negative correlation between GGT and ALP levels. Both GGT and ALP are enzymes involved in the induction and evaluation of diseases, with greater accuracy within cellular biliary duct alterations. Although GGT is less sensitive and can be considered more specific for dogs, it has lower sensitivity than ALP, with the latter being the most indicated enzyme for joint measurements (Allison 2015, Lawrence & Steiner 2017). An increase in these enzymes is expected in cases of acute liver damage caused by the induction of canaliculi. Noguchi et al. (2002) evaluated the possible hepatotoxic effects of copaiba oil in rats and observed the same negative effect in GGT measurements. Although they stated that, for rats,

the evaluation of the enzyme was inefficient for diagnosing cholestasis, they justified the observation by the decrease in mitochondrial oxygen uptake and, consequently, enzymatic inhibition (Freire et al. 2008).

Both albumin and thrombopoietin are synthesized by the liver tissue. The inverse correlation of albumin and platelet count values with the increase in the AST/ALT ratio is related to low metabolism by hepatocytes, tending to chronicity. Pohl et al. (2001) and Du et al. (2019) confirmed in their studies that lower platelet counts are related to an increase in the AST/ALT ratio. Values equal to or above 1.0, associated with platelet count lower than $150x10^3$/μL, were sensible to predict the diagnosis of severe fibrosis and advanced stages in human cirrhosis (Pohl et al. 2001, Du et al. 2019). Although this analysis was not made in the present study, opposite extremes could be observed in the multivariate analysis, reinforcing the importance of platelet evaluation in individuals with liver diseases.

Hepatopathies are often observed in canine clinical presentations because they can be correlated to any lesion that hepatocytes may pass through, including hypoxia, poisoning, metabolic diseases, inflammation, neoplasia, obstructions, considered direct injury, or even secondary affections (Allison 2015). With social family changes and the consequent inclusion of dogs and cats as family members, animals are living more, and chronic diseases such as cancer and renal alterations may lead to more cases for clinical care (Modiano & Breen 2007). Besides that, the presence of a specialized oncology team at the Veterinary Hospital in UFMG may explain the large number of neoplasm cases included.

In previous studies, female dogs diagnosed with cystic endometrial hyperplasia/pyometra showed AST/ALT ratios of up to 2.0 (Schepper et al. 1987). This phenomenon may be explained by bacterial transmigration and *Escherichia coli* toxins that act in the liver, leading to hepatotoxicity, as mentioned by Marinaska & Crovador (2019). In their study, authors observed that ALT presented lower serum activities represented by the average of 37.10±45.9UI/L (RV: 0-102UI/L), categorically within the reference values for the species. In contrast, higher values of AST were seen (an average of 54.3±44.8UI/L), some surpassing the reference value (0-66UI/L).

One of the main functions of the liver is the metabolism, detoxification, and excretion of harmful agents. According to Allison (2015), hepatotoxic substances may lead to severe injury to the liver parenchyma and may justify the findings of the present study correlated to higher values in the Intoxication/poisoning group.

An increase in Trauma/fracture group ratio may be correlated to muscular isoenzyme AST activity, as seen in cases of human rhabdomyolysis (Botros & Sikaris 2013). In cases of doubt, serum measurement of creatine phosphokinase (CK) should be performed, as recommended by Silva (2015) and Cerqueira (2017). In this study, this was not performed because CK is an analyte required separately from the routine biochemical profile in our routine, and too many results included it.

Considering leishmaniosis, this protozoan migrates to lymphoid organs, such as the liver and spleen, for proliferation, and the defense system represented by the macrophage tries to destroy it, opsonizing amastigotes. However, this process can damage the cells of the affected organs (Goto & Prianti 2009). The increase in lesion enzymes found in the Leishmaniosis group represents active lesions originating from the body's defense system against *Leishmania* infection in the liver.

The average AST/ALT ratio found in the Nephropathy group was 1.27±0.88. The same was observed by Zygner et al. (2012), with higher values observed in azotemic dogs infected with *Babesia canis* than in non-azotemic dogs. Although not specific to the renal system, the transaminase ratio, probably due to the influence of renal AST, may be useful for monitoring the progression of these patients. This study confirms that renal damage leads to an increase in AST (Zygner et al. 2012).

Aminotransferases in energetic metabolism are produced by many cancers and non-cancer cells (Bezan et al. 2015). Cancer cells perform their energetic metabolism, preferably via glycolysis using aspartate to metabolize in oxaloacetate. This condition leads to more activation of AST than ALT in cancer tissues with rapid growth (Dang 2012). The activities of AST and ALT can be influenced by changes related to the metabolism of cancer cells due to the facility to measure them and prove useful as prognostic biomarkers in these cases.

Most analyzed groups had high AST/ALT ratios. Generally, in medicine, higher values are associated with an unfavorable prognosis (Bezan et al. 2015, Gao et al. 2017, Zinellu et al. 2021). However, care should be taken in response to the disease profiles. Serial analysis of liver enzymes and the AST/ALT ratio, compared to cases with revaluation possibility, showed the importance of acute liver damage.

Attention must be paid to the kinetic activity of AST and ALT. Isolated acute lesions are characterized by increases in both enzymes, with a gradual reduction in the half-life corresponding to each aminotransferase in isolation. Canine AST has a half-life of up to 12 hours. When measured in parallel, its constancy, or even an increase, indicates active hepatocyte damage. ALT has a half-life of 2-3 days, and it is not possible to confirm whether an increase in its activity is present or present in the past (Stockham & Scott 2011).

Although ALT increase activity was present in some cases, even higher than and outside the reference values for dogs, AST was significantly reduced in some cases. From all the analyzed groups, it was possible to establish serial measurements at 48 h in only two groups: Pyometra and Intoxication/poisoning. Both diseases lead to acute liver damage owing to toxemia. Lesion enzymes tend to be elevated by active lesions in the liver tissue (Lawrence & Steiner 2017). After effective treatment and stabilization of patients, biochemical analyses performed after 48 h revealed a reduction in the relationship and clinical improvement. Thus, AST/ALT ratio values that remain high or increase in sequential measurements should more accurately predict the clinical course of the disease or even the effectiveness of the treatment when analyzed individually.

Considering prognosis, in all analyzed literature, values up to 1.0 were found to be correlated with unfavorable prognosis and risk of death in humans (Bezan et al. 2015, Gao et al. 2017, Zinellu et al. 2021). In veterinary medicine, no studies have investigated the prognostic value of the AST/ALT ratio in dogs.

Although the cutoff point for the worst prognosis of the ratio is 1.0 for humans, in the canine species, this value can be found in some healthy animals (Table 2) because the proportion of enzymatic activities is equivalent, with a slight trend of higher

ALT activities in the species, as stated Allison (2015). Higher ratios have been observed in chronic liver disease processes, whose prognosis is known to be worse. Since an exact cutoff point cannot be determined, the most recommended way to establish a prognosis is a serial evaluation of the enzyme activities (Lawrence & Steiner 2017). This measurement is simple and inexpensive. In this study, serial evaluation of the AST/ALT ratio was more useful for predicting prognosis than when the enzymes were analyzed separately. When the AST/ALT ratio doubled, there was a 2.5-fold increase in the chance of death (95% confidence interval), predicting that overcoming systemic diseases was worse than local processes.

CONCLUSION

The aspartate transaminase/alanine transaminase (AST/ALT) ratio distinguished animals with systemic diseases, including chronic hepatopathy, from healthy animals. Serial determination is important to evaluate the efficiency of therapeutic conduct; the greater the AST/ALT ratio, the greater the risk of death. Thus, the AST/ALT ratio should be included in the clinical routine and used to evaluate the evolution of both hepatic and extrahepatic diseases in dogs.

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