Serum lactate dehydrogenase activity in canine malignancies

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SERUM LACTATE DEHYDROGENASE ACTIVITY IN CANINE MALIGNANCIES

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Abstract

LDH is commonly used in human cancer patients for prognostic purposes. Aim of this study was to determine the magnitude of serum LDH elevation in dogs with cancer compared to healthy dogs and dogs with non-neoplastic disease, and to verify whether it may support the diagnosis of specific malignancies. 128 healthy dogs, 211 diseased dogs and 188 cancer dogs were enrolled. Dogs with cancer had significantly higher LDH than diseased (P<0.001) and healthy dogs (P<0.001), but large overlap was found. Dogs with lymphoma showed significantly higher LDH compared to dogs with carcinoma (P<0.001) or mast cell tumor (MCT; P<0.05) but not compared to other malignancies. When considering lymphoma and MCT, LDH levels were not different between early and advanced clinical stages. Measuring LDH levels may not be useful as a screening tool for cancer detection. More studies are needed to define its role in specific tumors.

Keywords
LDH, tumor marker, lymphoma, dog

Introduction

Lactate dehydrogenase (LDH), which is expressed as 5 isoenzymes, is an ubiquitous glycolytic enzyme catalysing the final step in the glycolytic metabolism, by converting lactate to pyruvate thereby producing cellular energy. In people, increased LDH activity has been found in patients with myocardial or pulmonary infarction, hepatic disorders, haemolysis, and myopathy. Additionally, LDH is one of the most common
enzymes used in cancer patients for prognostic purposes, as its increase reflects tumor burden and treatment response. Indeed, LDH is considered as an important prognostic marker in lymphoma, leukemia, germ cell tumors, and melanoma among others. Interestingly, serum measurement of LDH also helps in differentiating cancer from benign neoplasia, including uterine leiomyosarcoma from leiomyoma, and ovarian carcinoma from benign epithelial ovarian tumors.

In veterinary medicine, early detection of cancer is often challenging, partly due to the lack of adequate tumor markers. Thus, the diagnosis of cancer relies on tissue biopsy in the majority of cases. By definition, tumor markers are biologic substances produced by neoplastic cells or by the host in response to the cancer; they are typically secreted into bloodstream, urine, stool and other body fluids, and can be detected by several methods. Measuring tumor marker levels in addition to routine diagnostic tests may be useful to identify some types of cancer. Apart from diagnosis, tumor markers may also reflect the extent of cancer, predict the response to therapy, or facilitate the identification of recurrence during follow-up.

Little research about LDH and cancer has been carried out in veterinary medicine. According to the literature, LDH increases in canine and feline lymphoma, the pathogenesis of which remains undetermined. For other specific tumors, including carcinomas and sarcomas, information are not yet available. Although occasionally a higher specificity of LDH in neoplasias is found if the isoenzymes are assessed separately, total LDH measurement is sometimes preferred. While total LDH levels generally reflect presence of tissue damage, measurement of individual isoenzymes may give a clue on which organ or tissue is damaged.
Aim of this study was to determine whether elevation of total LDH occurred in dogs with cancer compared to healthy dogs and dogs with non-neoplastic disease, and to evaluate if the enzyme may be useful in supporting the diagnosis of specific malignancies.

**Materials and methods**

**Selection of dogs**

Healthy dogs presenting for periodical examination (including general health check and/or regular vaccination), untreated dogs with various non-neoplastic diseases, and untreated dogs with cancer, admitted to the Clinica Veterinaria L’Arca, were prospectively enrolled.

For each included case, signalment information was obtained. Dogs with non-neoplastic diseases were presented with a variety of clinical signs; they had no evidence of malignancy based on results of physical examination, blood work, thoracic radiography and/or abdominal ultrasound.

All cancer cases were diagnosed by means of cytology and flow cytometry (liquid tumors) or histopathology (solid tumors) and underwent complete staging work-up, including thoracic radiology, abdominal ultrasound, and evaluation of the regional lymph node. For hematopoietic cancer, histiocytic disease and mast cell tumors, cytological samples of the bone marrow were also obtained.

**LDH evaluation**

At presentation, blood from the jugular vein was sampled after 12-hours fasting, and the serum obtained by centrifugation was processed within 30 minutes.
Given the fact that all kind of tumors could be enrolled, either localized and metastatic, total serum LDH measurement was preferred over isoenzymes. For all dogs, total LDH levels were determined with a commercial assay (kinetic UV method Pyruvate-Lactate, Biogamma SRL, Rome, Italy) according to the manufacturer. The normal range of total serum LDH at our laboratory is 10-280 U/l. For each included dog, the following haematological parameters were also recorded: packed cell volume (PCV), albumin, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

**Statistical analysis**

Comparison among LDH levels in dogs with cancer and healthy or diseased dogs was calculated with one-way ANOVA followed by Tukey’s multiple comparison test. The same analysis was used to compare LDH levels among specific tumors; types of cancer were included if represented by at least 10 cases. To assess whether tumor stage, which generally reflects cancer extension, is associated with LDH levels, concentrations were compared between stages for selected tumors, including lymphoma and mast cell tumor with the T-test or Mann-Whitney test. For lymphoma cases, comparison was made between stage I-IV and stage V. Stages I-IV were grouped together because biologically less aggressive than stage V (meaning bone marrow involvement). For mast cell tumors, comparison was made between stage II and IV grouped together (metastatic to regional lymph nodes and distant sites, respectively) and stage I and III (non metastatic). Because LDH levels may be affected by several factors in humans, including anemia, liver disease and renal failure, possible relationships between LDH levels and laboratory parameters, such as PCV, albumin, creatinine, ALT, AST or ALP, were investigated in all dogs with Pearson correlation coefficient. Because aging dogs are more likely to be affected by a disease, the correlation was also investigated between
LDH levels and age. Significance was considered for p < 0.05. Statistical analysis was performed with GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA, USA).

Results

Healthy dogs

One-hundred twenty-eight healthy dogs were enrolled. Median age was 7 years (range, 0.3 to 18 years). There were 83 males (69 intact, 14 neutered) and 45 females (23 intact, 22 spayed). Regarding breed, there were 58 mixed breeds and 70 pure breeds, with German shepherd dogs (11/70, 15.7%), Labrador retrievers (9/70, 12.9%) and Yorkshire terriers (6/70, 8.6%) being more frequent.

At presentation, mean LDH level was 142.3 ± 79.5 U/l (range, 22 to 523 U/l). One-hundred twenty (93.8%) dogs had normal LDH, whereas 8 (6.2%) dogs had elevated LDH.

Dogs with non-neoplastic disease

Two-hundred eleven diseased dogs were enrolled. Median age was 9 years (range, 0.1 to 18 years). There were 114 males (101 intact, 13 neutered) and 97 females (51 intact, 46 spayed). Regarding breed, there were 97 mixed breeds and 114 pure breeds, with Yorkshire terriers (20/114, 17.5%), Labrador retrievers (17/114, 14.9%), German shepherd dogs (16/114, 14.0%) and Cocker spaniels (7/114, 6.1%) being more frequent.

The most common disorders were: urogenital diseases (n=28), gastroenteritis (n=25), dermatologic diseases (n=22), leishmaniosis (n=19), hepatopathies (n=15), cardiac diseases (n=13), arthrosis (n=11), epilepsy (n=8), diabetes mellitus (n=7), ehrlichiosis
(n=7), immune-mediated haemolytic anemia (n=6), hydrocephalus (n=3), hypothyroidism (n=3), pancreatitis (n=3), hemophagocytic syndrome (n=3), systemic inflammatory response syndrome (n=3), tracheal collapse (n=2), reactive histiocytosis (n=2), hypovolemic shock (n=2).

When considering specific diseases, dogs with pyometra had more often elevated LDH levels (6/9, 66.7%). Unlike previous experience in human medicine, here only 5 (38.5%) out of the 13 dogs with cardiac diseases and 5 (33.3%) out of the 15 dogs with hepatopathies had increased LDH levels.

Among dogs with leishmaniosis and ehrlichiosis, only 2 out of 19 (10.5%) and 1 out of 7 (14.3%), respectively, had increased LDH levels at presentation. All of them showed generalized peripheral lymphadenopathy, a clinical finding which is typical of multicentric lymphoma as well.

At presentation, mean LDH level was 232.8 ± 202.9 U/l (range, 33 to 1800 U/l). One-hundred sixty-two (76.8%) dogs had normal LDH, whereas 49 (23.2%) dogs had elevated LDH.

Dogs with cancer

One-hundred and eighty-eight dogs with cancer were included. Median age was 10 years (range, 0.6 to 18 years). There were 96 males (87 intact, 9 neutered) and 92 females (47 intact, 45 spayed). Regarding breed, there were 76 mixed breeds and 112 pure breeds, with Rottweilers (14/112, 12.5%), German shepherd dogs (11/112, 9.8%), Yorkshire terriers (9/112, 8.0%), Labrador retrievers (8/112, 7.1%), cocker spaniels and boxers (6/112, 5.4%, each) being more frequent.

The following tumors were diagnosed: lymphoma (n=55), mammary carcinoma (n=17), mast cell tumor (n=17), histiocytic sarcoma (n=11), pituitary dependent hyperadrenocorticism (n=11), hemangiosarcoma (n=10), osteosarcoma (n=9), soft tissue
spindle cell tumors (n=6), mammary inflammatory carcinoma (n=4), prostatic
carcinoma (n=3), tonsilar carcinoma (n=3), chronic lymphocytic leukemia (n=3), Sertoli
cell tumor (n=3), hepatocellular carcinoma (n=2), perianal carcinoma (n=2), digital
squamous cell carcinoma (n=2), bladder urothelial carcinoma (n=2), acute
lymphoblastic leukemia (n=2), intestinal leiomyosarcoma (n=2), oral fibrosarcoma
(n=2), carcinoid (n=2), adrenal dependent hyperadrenocorticism (n=2), and one each of
the following: bladder adenocarcinoma, perianal adenoma, bronchioloalveolar
carcinoma, renal carcinoma, anal sac carcinoma, nasal carcinoma, oral squamous cell
carcinoma, aortic chemodectoma, cholangiocarcinoma, gastrinoma, Leydig cell tumor,
histiocytoma, oral melanoma, digital melanoma, multiple myeloma, thymoma,
meningioma, and venereal transmissible tumor.

At presentation, mean LDH level for all cancer cases was 341.6 ± 342.5 U/l (range, 13
to 3325 U/l). One-hundred and six (56.4%) dogs had normal LDH, whereas 82 (43.6%)
dogs had elevated LDH.

Regarding lymphoma cases, mean LDH level was 511.1 ± 450.9 U/l (range, 86 to 3325
U/l). Among them, 43 of 55 (78.2%) dogs had elevated LDH levels at diagnosis. When
considering clinical stage, dogs with stage I-IV (n = 32) disease had mean LDH level of
430.8 ± 450.9 U/l (range, 86 to 1132 U/l), whereas dogs with stage V (n = 23) disease
had mean LDH level of 622.7 ± 633.7 U/l (range, 120 to 3325 U/l).

Regarding mast cell tumors, mean LDH level was 205.6 ± 210.2 U/l (range, 40 to 817
U/l). Among these cases, 3 of 17 (17.6%) had elevated LDH at presentation. When
considering clinical stage, dogs with stage I and III disease (n = 11) had mean LDH
level of 175.6 ± 162.4 U/l (range, 72 to 644 U/l), whereas dogs with stage II and IV
disease (n = 6) had mean LDH level of 260.7 ± 288.1 U/l (range, 40 to 817 U/l).

Regarding dogs with histiocytic sarcoma, mean LDH level was 344.4 ± 247.3 U/l
(range, 73 to 693 U/l). Among them, 5 of 11 (45.5%) had elevated LDH at presentation.
Comparison among groups

Mean LDH concentration was significantly higher in tumor-bearing dogs than either healthy dogs or dogs with non-tumoral diseases. The latter group had significantly higher levels than healthy dogs (fig. 1).

Considering specific tumors, mean LDH levels were significantly higher for dogs with lymphoma than either dogs with carcinoma or mast cell tumor (Figure 2). Differences were not observed with sarcoma, histiocytic sarcoma, and hyperadrenocorticism.

LDH levels were similar between dogs with lymphoma stage V and I-IV, or between dogs with mast cell tumor stage II or IV and I or III.

Considering the whole population of dogs, a significant positive correlation was found between LDH levels and age ($r= 0.15; CI95\% = 0.07 - 0.24$), and a negative correlation between LDH and PCV ($r= -0.10; CI95\% = -0.18 - -0.02$) or albumin ($r= -0.18; CI95\% = -0.26 - -0.10$) (Figure 3). Correlations were not found with ALT, AST or ALP (data not shown).

Discussion

Early detection of cancer is still very challenging. Beside ultrasound, CT scan, magnetic resonance, and newer imaging techniques, such as positron emission tomography, are used for tumor staging and are considered helpful for early diagnosis. Despite progresses, the minimum tumor size enabling detection with the afore-mentioned techniques is represented by $10^9$ cells, a point at which successful intervention is often not possible. Little data has been published in the veterinary literature on circulating diagnostic tumor markers, including acute phase proteins, alpha-fetoprotein, alkaline
phosphatase, thymidine kinase, and lipid-associated sialic acid, yet their biologic significance has not been completely elucidated.

Determination of serum LDH levels is a well-known diagnostic tool in human oncology, and occasionally, measurement of LDH levels failed to show specificity in human cancer patients. For instance, high LDH levels in melanoma patients do not necessarily indicate a more advanced disease, thereby possibly leading to false-positive results if not cautiously interpreted during initial staging work-up.

High LDH levels were also reported in dogs and cats with lymphoma. In this study, we assessed LDH levels in dogs with various types of cancer. Values recorded in dogs considered to be clinically healthy and in those affected by non-neoplastic diseases served as controls. Dogs with cancer were found to have significantly higher serum LDH levels than dogs in the healthy control group (P<0.001) and dogs with non-neoplastic diseases (P<0.001). Altogether, LDH was found to be increased in 43.6% of cancer cases. Among them, dogs with lymphoma had more often elevated LDH levels (78.2%) at presentation. Levels of LDH were higher in lymphoma than in either carcinoma (P<0.001) or mast cell tumors (P<0.05).

Although mean LDH levels were significantly higher in cancer patients (341.6 ± 342.5 U/l) than in dogs with non-neoplastic disease (232.8 ± 202.9 U/l) or in healthy individuals (142.3 ± 79.5 U/l), considerable overlap existed between groups, likely preventing its use as useful marker for tumor detection. In addition, about 50% of cases with proven malignancy had no LDH elevation at presentation, whereas 23.2% of dogs with non-neoplastic disease and 6.2% of healthy dogs had increased LDH, thereby indicating that a cut-off point discerning tumor-bearing negative and positive cases cannot be identified.

Also in the case of tumor types, LDH levels were largely overlapping. Thus, measuring activity of the enzyme has limited usefulness, especially in tumor-bearing dogs with
normal LDH levels or when a tumor has been confirmed but its origin has not been clarified.

We also analyzed the role of LDH in different stages of lymphoma and mast cell tumors (early versus metastatic). Its level was not found to be different between lower and higher stages. It is possible that, for mast cell tumors, the low proportion of metastatic cases (6/17; 35.3%) prevented a significant difference to emerge. The contrary may hold true for lymphoma, since there were no early stage (I-II) lymphoma cases. Alternatively, LDH is not useful in these patients to differentiate between metastatic/advanced and non-metastatic/early cancer.

To the authors’ knowledge, there are no studies exploring the diagnostic utility of LDH in differentiating benign from malignant disease. Beside multicentric lymphoma, further pathological conditions frequently associated with generalized lymphadenopathy include leishmaniosis, ehrlichiosis, bacterial, viral and fungal infections. Cytological evaluation of the enlarged lymph nodes by a skilled clinical pathologist leads very often to the diagnosis of lymphoma. Routine blood work, including assessment of serum globulins, may provide additional valuable diagnostic information. In this case series, there were 19 dogs with leishmaniosis and 7 dogs with ehrlichiosis, all presenting with enlargement of the peripheral lymph nodes. An interesting finding was that 78.2% of dogs with lymphoma had increased LDH levels, whereas only 10.5% and 14.3% of dogs with leishmaniosis and ehrlichiosis, respectively, showed increased concentration. Thus, LDH measurement may be useful in the initial work-up in selected cases, more likely suggesting a malignant etiology if increased.

Not unexpected, a correlation was found between age and LDH levels, likely due to the fact that older dogs are more likely to develop diseases. The negative correlation observed with PCV or albumin concentrations may suggest a shift towards anaerobic
glycolysis in tissues, at least in part due to hypoxemia. However, it must be noted that in each instance correlations were weak, questioning the value of the findings.

In conclusion, this study showed that LDH levels has limited use in differentiating dogs with cancer from healthy dogs and dogs with non-neoplastic disease, or among different tumors, because results largely overlap. Furthermore, in dogs with lymphoma and mast cell tumors, LDH does not help identifying early or advanced stage cancers. In selected cases, such as in dogs with enlarged lymph nodes due to lymphoma or leishmaniosisis and ehrlichiosis, knowing LDH levels may help refining the diagnosis. More studies are needed to definitely clarify the role of LDH in dogs with lymphoma.

References


Abbreviated title: LDH and cancer
Figure legends

**Figure 1.** Serum levels of LDH measured in clinically healthy dogs, and dogs with non-tumoral disorders or with tumor. Scatter plot and mean values are reported.

**Figure 2.** Serum levels of LDH measured in dogs with different tumors, including carcinoma, sarcoma, lymphoma, mastocytoma, histiocytic sarcoma and hyperadrenocorticism. Values are expressed as mean and standard deviation.

**Figure 3.** Correlation between serum levels of LDH and age (A), PCV (B), and albumin concentration (C) in all dogs. Regression lines are plotted.
Figure 1

LDH (U/l)

Healthy Non-tumor Tumor

P<0.01

P<0.001

P<0.001
Figure 3

A

P < 0.001

B

P = 0.026

C

P < 0.0001