Diagnostic utility of measuring lactate dehydrogenase levels and its isoenzyme activities for the evaluation of malignancy in feline pleural effusion and ascitic fluid

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Short Report

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Abstract

Lactate dehydrogenase (LDH) isoenzymes may be useful in the differential diagnosis of pleural effusion (PE) and ascitic fluid (AF) etiologies in cats since tissue damage induces their release, thereby changing the pattern of their activity. The present study aimed to determine the diagnostic utility of measuring LDH levels and its isoenzyme activities in PE or AF in cats with malignancy. We measured LDH levels and its isoenzyme activities in serum, PE, and AF in 29 cats and compared the results between malignant, infectious diseases, and non-malignant, non-infectious diseases. Receiver operating characteristic (ROC) analysis was performed to assess the accuracy of the tests in diagnosing feline malignancy. In PE or AF, significant differences were found in LDH levels and LDH isoenzymes activities among the three groups. The combination of LDH level and LDH-1 activity in PE or AF had the highest area under the ROC (AUC) value of 0.874 for discriminating malignant effusion from non-malignant effusion. The sensitivity and specificity of using the combination of LDH level (cutoff: <2,269 U/L) and LDH-1 activity (cutoff: <4.8%) in PE or AF for predicting malignancy with the highest AUC value were 94.4% and 72.7%, respectively. Our results suggest that the combination of LDH level and LDH-1 activity in PE or AF can potentially diagnose for malignancy. Considering that LDH isoenzymes can be measured inexpensively and easily, LDH tests can be readily accommodated in veterinary clinical practice.

Introduction

Pleural effusion (PE) and ascitic fluid (AF) can present interesting diagnostic challenges in cats. Differential diagnoses related to PE and AF in cats could include a variety of diseases (Ettinger et al. 2017). However, the classification of PE and AF into malignant or non-malignant remains challenging, despite using various tests.

In humans, lactate dehydrogenase (LDH), which plays a role in the glycolytic way, has been the focus in the differential diagnosis of various diseases because of their release from cells after cell damage or death (Dawson et al. 1964). The distributions of LDH five isoenzymes differ in various tissues (Lott and Stang 1980; Wroblewski and Gregory 1961). However, the diagnostic values of serum LDH level and LDH isoenzyme patterns are still controversial (Dawson et al. 1964; Wroblewski and Gregory 1961). In contrast, LDH isoenzyme activities in PE and AF are useful biomarkers for distinguishing neoplastic and non-neoplastic diseases (Cobben et al. 1997; Lossos et al. 1999; Paavonen et al. 1991; Sevinc et al. 2005). Interestingly, LDH-1 activity, which works efficiently under aerobic conditions, is significantly lower in neoplastic diseases than in non-neoplastic diseases, whereas LDH-4 level, which works efficiently under anaerobic conditions, is significantly higher in neoplastic diseases than in non-neoplastic diseases (Paavonen et al. 1991; Sevinc et al. 2005).

In veterinary medicine, serum LDH level in cats with lymphoma are a potential negative prognostic indicator of survival (Hadden et al. 2008). Also, LDH level in PE in cats with feline infectious peritonitis (FIP) and pyothorax were increase (Zoia et al. 2009). However, few reports have been made regarding LDH isoenzyme analyses in feline PE and AF.

The present study evaluated LDH level and LDH isoenzyme activities in serum, PE, and AF in cats with neoplastic and non-neoplastic diseases. We hypothesized that LDH level and anaerobic LDH isoenzyme activities in PE and AF with neoplastic and infectious diseases would be higher than that with other diseases.

Materials And Methods

This retrospective cross-sectional study analyzed cats in which PE or AF was newly detected at the Yuki Animal Hospital from December 2014 to December 2018. These cats were divided into 2 groups based on diagnosis. The malignant group included cats with tumors that were diagnosed by the detection of tumor cells by fine needle aspiration, clonality analysis, or biopsy. The non-malignant group included cats with diseases that cause PE and AF without tumors. The non-malignant group included two subgroups: non-malignant group 1: non-infectious diseases such as idiopathic chylothorax, congestive heart failure (CHF), and liver disease; and non-malignant group 2: infectious diseases such as FIP, pyothorax, and bacterial peritonitis. CHF was confirmed by cardiac ultrasonography. Liver disease was diagnosed by histopathological examination of the liver. FIP was diagnosed when supportive history, signalment, and clinical and clinicopathological data were present and no other causes of PE and AF could be identified (Zoia et al. 2009). Pyothorax and bacterial peritonitis were diagnosed by positive bacterial culture. The exclusion criteria of this study were cats with an incomplete diagnosis and cats with co-existing diseases that cause PE and AF. All serum, PE and AF samples were centrifuged and separated within 30 min. All the samples were stored at 4°C during the experiment. The LDH level and LDH isoenzyme activities were measured in all samples (LSI Medience Co., Tokyo, Japan). The results of each laboratory test and the ages of the cats were compared among the malignant group, non-malignant group 1, and non-malignant group 2 using Kruskal–Wallis test. Post hoc comparisons were conducted using Mann–Whitney *U* test with Bonferroni correction. The accuracy of the tests between the malignant and non-malignant groups was analyzed by receiver operating characteristic (ROC) analysis. The area under the ROC curve (AUC) and sensitivity and specificity with 95% confidence intervals (CI) were calculated. Logistic regression was performed to evaluate the diagnostic accuracy of malignancy by the combination of the LDH level and LDH isoenzyme activity used for ROC analysis. A *P* value of <0.05 was considered significant. Statistical analyses were performed using Easy R software (Kanda 2013).

Results

Forty-nine cats were newly diagnosed with PE or AF. The malignant group included 11 cats, 5 with PE and 6 with AF. The cats with PE were diagnosed with the following diseases: 3 with lung carcinoma, 1 with mediastinal lymphoma, and 1 with extranodal lymphoma. The cats with AF were diagnosed as follows: 3 with alimentary lymphoma, 1 with renal lymphoma, 1 with intestinal carcinoma, and 1 with hepatocellular carcinoma. Non-malignant group 1 included 12 cats, 10 with PE and 2 with AF. The cats with PE were diagnosed as follows: 7 with hypertrophic cardiomyopathy, 1 with dilated cardiomyopathy, 1 with restrictive cardiomyopathy, and 1 with unclassified cardiomyopathy. The cats with AF were diagnosed as

follows: 1 with hypertrophic cardiomyopathy by ultrasonography and 1 with cholangitis and hepatitis by histopathological liver examination. Non-malignant group 2 included 6 cats, 2 with PE and 4 with AF. Two cats with PE were diagnosed with FIP. Three cats with AF were diagnosed with FIP, and 1 cat was diagnosed with bacterial peritonitis. Twenty cats were excluded.

The cat breeds included 25 crossbreeds, two Scottish Folds, and two Ragdolls. The malignant group consisted of 6 males (2 castrated) and 5 females (3 spayed). The median age was 12.2 years (range, 3–17.4 years). Non-malignant group 1 consisted of 6 males (4 castrated) and 6 females (4 spayed). The median age was 16 years (range, 6.8–21.4 years). Non-malignant group 2 consisted of 5 males (2 castrated) and 1 intact female. The median age was 3.8 years (range, 0.4–17.0 years).

Data of LDH and LDH isoenzymes were shown in Table and Figure 1. In serum, there were no significant differences in LDH level and LDH isoenzymes activities among 3 groups. In PE and AF, the LDH level in the malignant group (median, 441 U/L; interquartile range [IQR], 151–1,016 U/L) was significantly higher than that in the non-malignant group 1 (median, 65 U/L; IQR, 47–93 U/L) ($P = 0.007$), and lower than that in the non-malignant group 2 (median, 2,477 U/L; IQR, 2,284–2,928 U/L) ($P = 0.015$). The LDH-1 activity in the malignant group (median, 4.4%; IQR, 3.5%–6.0%) was significantly lower than that in non-malignant group 1 (median, 29.6%; IQR, 23.4%–44.5%) ($P < 0.001$), and higher than that in non-malignant group 2 (median, 2.0%; IQR, 1.3%–2.7%) ($P = 0.031$). The LDH-2 activity in non-malignant group 1 (median, 15.9%; IQR, 12.0%–17.1%) was significantly higher than that in non-malignant group 2 (median, 6.0%; IQR, 4.5%–7.0%) ($P = 0.004$). The LDH-3 activity in the malignant group (median, 20.4%; IQR, 20.1%–25.0%) was significantly lower than that in the non-malignant group 1 (median, 10.1%; IQR, 9.5%–12.5%) ($P = 0.007$). The LDH-4 activity in the malignant group (median, 31.8%; IQR, 29.5%–34.7%) was significantly lower than that in the non-malignant group 1 (median, 16.8%; IQR, 12.9%–23.9%) ($P = 0.002$), and lower than that in the non-malignant group 2 (37.6%; 36.2%–39.4%) ($P = 0.015$).

The results of the ROC curve analysis are shown in Table. The combination of LDH level and LDH-1 activity in PE or AF showed the highest AUC value for discriminating malignant effusion from non-malignant effusion compared with other factors. The AUC of the combination of LDH level and LDH-1 activity in PE or AF was 0.874 (95% CI: 0.739–1.000), with a sensitivity and specificity 94.4% and 72.7%, respectively (cutoff: LDH, <2,269 U/L; LDH-1 activity, <4.8%).

Discussion

The present study demonstrated the diagnostic utility of the LDH level and LDH isoenzyme activities in feline PE and AF. Especially, the combination of the LDH level and LDH-1 activity had the highest diagnostic potential in distinguishing malignancy from non-malignancy. In our study, neoplastic disease behaved similarly to infectious diseases in LDH level and LDH isoenzyme activities. Furthermore, infectious diseases tend to produce higher LDH level than other diseases, whereas infectious diseases tend to have lower LDH-1 activity than other diseases. Generally, cytological tests for malignant cells in PE and AF demonstrate high specificity (100%) but low sensitivity (50%) (Spangler et al. 2000). In the

present study, all cats in the malignant group did not be diagnosed by cytological testing with PE and AF; therefore, LDH isoenzyme analyses have a potential of diagnostic biomarker regardless of cytological testing with PE and AF.

In the present study, there were no significant differences in serum LDH level and LDH isoenzyme activities among 3 groups. By contrast, the LDH level and LDH isoenzyme activities in PE and AF differed significantly among neoplastic, infectious diseases and non-malignant, non-infectious diseases. These differences in LDH levels and LDH isoenzyme activities may be caused by differences between body fluids and blood. Saint-Rémy et al. (1986) reported that the LDH level and LDH isoenzyme activities might be affected by the release of enzymes from inflammatory cells and erythrocytes present in body fluid. Paavonen et al. (1991) reported that visceral or parietal pleural cells are rich in LDH. For these reasons, Sevinc et al. (2005) deemed that LDH isoenzymes might be released from cells that infiltrate body fluids other than serum. Thus, the LDH level in feline body fluids may be higher than in serum, similar to that in humans, and the differences between body fluids and serum are easily observed.

LDH is present in essentially all organ systems and is released from cells only after cell damage or death (Drent et al. 1996; Glick et al. 1969). Elevated LDH levels in PE or AF were caused by activated, injured, or dead leukocytes, tumor cells, or mesothelial cells (Sevinc et al. 2005; Washizu et al. 2002). The LDH level depends on the number of cells, such as leukocytes, tumor cells, or mesothelial cells in PE and AF, because LDH is released from cells. Moreover, LDH is also released from dead cells, regardless of the number of cells in PE and AF. Therefore, significant differences were observed among 3 groups in the present study due to the difference in the origin of LDH.

This study demonstrated that LDH-1 activity in PE or AF increased in cats with CHF and liver disease. In humans, CHF increases the percentile of LDH-1 activity in PE and AF due to aerobic conditions in heart tissue (Firth et al. 1995; Cobben et al. 1997; Sevinc et al. 2005). Increasing in LDH-1 activity in AF was also observed in patients with chronic liver disease (Sevinc et al. 2005). These findings were reflected in the diagnoses of the cases in the present study. In addition, LDH-3 and LDH-4 activities increased in the PE or AF of cats with neoplastic and infectious diseases. Tumor cells need LDH to maintain and increase (Fantin et al. 2006). Hypoxic areas in malignancy have been shown to increase (Fantin et al. 2006). Human LDH-4 activity in PE and AF were found to be significantly higher in malignancy than in non-malignancy (Cobben et al. 1997; Sevinc et al. 2005). These studies included patients with non-blood cancers. Whereas, the malignant group in the present study included 6/11 cats with lymphoma. Anaerobic LDH isoenzymes have been reported the most abundant isoenzyme in lymphoid tissue (Dumontet et al. 1999). Moreover, leukocytes have more anaerobic LDH isoenzymes in cats (Washizu et al. 2002). These results suggested that the distribution of LDH-3 and LDH-4 in neoplastic and infectious group in cats were increase.

This study investigated PE and AF together. Generally, LDH isoenzymes content vary across different organs. However, in our research, no significant differences in LDH level and LDH isoenzyme activities were found between PE and AF in each of the malignant and non-malignant groups (data not

shown). Therefore, we included cats with both PE and AF. The malignant group included various neoplastic diseases. The age, weight, and breed in each group were not consistent. Given that only examined cats were selected, our findings may not be reflective of all cats with malignancy.

In conclusion, the present study suggests that the combination of LDH level and LDH isoenzyme activities in PE or AF are potential factors for diagnosing malignancy.

Declarations

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Authors' Contributions

EN designed the study. EN, RS, and MY participated in sample collection. EN and RS collected and analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest: The test for LDH level and LDH isoenzyme activity measurements was provided by LSI Medience Corp. The study plan, data analyses, data interpretation, and decision to publish the results were not influenced by the funders.

Data Availability: The data that support the findings of this study are available from the corresponding author (M.Y.) upon reasonable request.

Ethical Approval: This work involved the use of non-experimental owned cats only. In Japan, there is no ethics committee available for private-practice animal hospitals. Nevertheless, this research was conducted according to the ethical codes of the Japan Veterinary Medical Association.

Consent to participate: The samples obtained in this study were used after obtaining consent from each cat owner.

Consent for Publication: Not applicable.

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Tables

Due to technical limitations, table 1 and 2 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Comparison of LDH isoenzyme activities in PE or AF between the malignant and non-malignant groups. Box plots show the median, range, and 25th and 75th quartiles within the groups. LDH level, LDH-3, and LDH-4 activities were significantly higher in the malignant group than in non-malignant group 1 ($P = 0.007$, 0.007 , and 0.002 , respectively). LDH-1 activity was significantly lower in the malignant group than in non-malignant group 1 ($P < 0.001$). LDH-1 activity was significantly lower in the malignant group than in non-malignant group 2 ($P = 0.031$). LDH level and LDH-4 activity were lower in the malignant group than in non-malignant group 2 ($P = 0.015$, respectively). Statistical analyses were performed using the Kruskal–Wallis test. Post hoc comparisons were conducted using the Mann–Whitney U test with Bonferroni correction. * $P < 0.05$; LDH, lactate dehydrogenase; PE, pleural effusion; AF, ascitic fluid.

Supplementary Files

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