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Lymphopenia predicts reduced survival in canine hepatocellular carcinoma

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ABSTRACT

Platelet to lymphocyte ratio (PLR) is a prognostic marker in human hepatocellular carcinoma (HCC) however, its utility in canine HCC has not been explored. The aim of the study was to determine if PLR could predict survival outcomes in 42 dogs with HCC. PLR was not a significant predictive factor ($p = 0.15$) but lymphopenia alone was significantly correlated with a reduced probability of survival ($p = 0.024$). Further studies are needed to evaluate if peripheral lymphocyte count mirrors that of the tumor microenvironment in canine HCC.

Keywords: Liver; cancer; dogs; carcinoma, hepatocellular; tumor microenvironment

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary hepatic cancer in dogs [1,2]. The prognosis for long-term survival has been previously correlated with several factors, including upregulation of liver enzyme activity (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], and gamma-glutamyl transferase [GGT]), and increase in blood urea nitrogen [BUN], calcium, potassium, and thrombocytosis [2-4]. Additional unfavorable prognostic indicators include tumor size > 5 cm, location of the tumor within the central and right divisions, and lack of surgical resection [2-4]. In human patients with HCC, biomarkers calculated from basic hematologic parameters are associated with prognosis [5,6].

Platelet to lymphocyte ratio (PLR) has been well documented as a negative predictor of survival in human patients with HCC [7,8]. It is postulated that an increased PLR reflects a pro-inflammatory tumor phenotype. However, use of the PLR in canine HCC has not been explored. The objective of this study was to describe the utility of PLR as a prognostic indicator in dogs with HCC. The secondary objective was to describe clinical and biochemical features of dogs with HCC and add to the cumulative data on survival characteristics in canine HCC.

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MATERIALS AND METHODS

Data collection

Records from the Veterinary Teaching Hospital at the Louisiana State University School of Veterinary Medicine (LSUVTH, IDEXX Cornerstone Medical Records Software, IDEXX Computer Systems, USA) and Louisiana Animal Disease Diagnostic Laboratory were searched for cases of hepatocellular carcinoma from 2005 to 2022. The search was conducted by using the key words "hepatocellular carcinoma" and "hepatic carcinoma." Study inclusion required a definitive histologic diagnosis of HCC. Forty-two dogs were identified.

Clinical data

Signalment, sex, breed, clinicopathological parameters (complete blood count, serum/plasma biochemical profile), and coagulation profile (prothrombin time, partial thromboplastin time) were recorded. The PLR was calculated. Serum biochemistry values such as blood glucose, AST, ALT, ALP, GGT, total bilirubin, albumin, cholesterol, BUN, phosphorus, sodium, potassium, and chloride were recorded. Hematologic, serum/plasma biochemistry, and coagulation abnormalities were defined based on the reference intervals established by the LSUVTH Clinical Pathology Laboratory.

Cytology

Cytology reports from dogs in which ultrasound-guided fine-needle aspiration of the mass was performed were reviewed. A diagnosis of hepatic neoplasm was recorded if the cytologic interpretation contained the following phrases: consistent with well-differentiated hepatocellular neoplasm or suspicion of well-differentiated HCC. Other interpretations were considered non-diagnostic.

Imaging

Abdominal ultrasound (AUS) and computed tomography (CT) reports were screened for tumor location, mass number, and tumor diameter. The location of the neoplasm on imaging was recorded as left liver (left lateral, left medial, and papillary process of caudate lobe), central liver (right medial and quadrate lobes, including the hilus), and right liver (right lateral and caudate process of the caudate lobe) [3].

Survival data

Overall survival was calculated from the time of the histologic diagnosis of HCC to the time of death. Survival outcomes were determined from the case records, consultation with the referring veterinarian or the owner. If date of death could not be determined, the patient was excluded from the survival analysis.

Statistical analysis

All data analyses were performed using JMP Pro 16.2.0 (SAS Institute Inc., USA). Heat maps were generated by standard score using GraphPad Prism version 9.4.1 for Windows (GraphPad Software, USA). The top four factors influencing survival categories: 0–6 mon, 6–12 mon, 1–2 yr, and over 2 yr were determined via random forest algorithm. PLR was analyzed with binary outcomes (less or more than 1 yr survival) with receiver operating characteristic (ROC) curve analysis. For survival analysis, Kaplan-Meier method was used for categorical variables and Cox proportional hazard regression models were used for continuous variables. A p value less than 0.05 was considered significant.

RESULTS

Patient demographics

Of the forty-two dogs with histologically confirmed HCC, the median age at diagnosis was 12.5 yr (range: 7.0–17.4 yr). Twenty-seven breeds were identified, the largest group was classified as mixed breed dogs (33.33%). The most common breeds included Labrador retrievers (9.52%), Jack Russell Terriers (7.14%), Catahoula Leopard Dog (4.76%), and Scottish Terriers (4.76%). Gender distribution included 24 (57.14%) spayed females, 15 (37.71%) neutered males, 2 (4.76%) intact females, and 1 (2.38%) intact male. At the time of HCC diagnosis, 6 patients (14.3%) had a second neoplasm. Survival data was available for 36 (85.7%) patients. Eight (19%) were still alive at the termination of data collection. Ten (23.8%) were euthanized due to progression of disease, 2 patients died from tumor-related causes (4.8%), and 1 (2.4%) patient died due a non-tumor-related cause. The overall median survival time (MST) was 358 d (range: 0–1,790 d). Age at death ranged from 7.0 to 19.1 yr (median: 14.0 yr).

Hematological and biochemical profile

Clinicopathologic findings are summarized in **Table 1**. The top four clinicopathologic and biochemical factors influencing survival intervals were hyperphosphatemia, lymphopenia, and increased activities of ALP and GGT (**Fig. 1**). Lymphopenia, present in 29.3% (12/41) of dogs, was significantly correlated with a reduced probability of survival ($p = 0.024$) with a risk ratio of 0.6721 per 1,000 units change (range: 0.4514–0.9369, $p = 0.016$, **Fig. 2**).

Table 1. Clinicopathological profiles

Characteristics	Median (range)	No. of dogs	Dogs with increased values (%)	Dogs with decreased values (%)	Reference interval
Hematology					
RBC (10^6/μL)	7.7 (2.7–8.6)	41	2.4	36.6	5.40–8.40
Platelets (10^3/μL)	426 (45–974)	41	26.8	9.8	220–600
WBC (10^3/μL)	11.5 (6.0–37.3)	41	31.7	17.1	8.0–14.5
Lymphocytes (10^3/μL)	1.3* (0.2–6.6)	41	2.4	29.3	1.00–4.80
PLR	334.6 (23.8–3,715.0)	41	78.0	19.5	
Biochemistry					
AST (U/L)	50 (13–1,083)	35	48.6	0	0–50
ALT (U/L)	310 (12–6,181)	38	89.5	0	0–60
ALP (U/L)	1,654 (29–8,570)	39	97.4	0	0–100
GGT (U/L)	11 (1–78)	27	59.3	0	0–8
Glucose (mg/dL)	106 (90–521)	40	35.0	0	80–115
Bilirubin (mg/dL)	0.3 (0.005–7.8)	39	12.8	0	0.0–0.4
Albumin (g/dL)	3.2 (1.6–4.2)	39	0	10.3	2.6–4.2
Chol (mg/dL)	285 (137–655)	33	69.7	3.0	150–240
BUN (mg/dL)	18 (8–78)	40	37.5	5.0	8–22
P (mg/dL)	3.9 (2.9–7.2)	35	5.7	20.0	3.4–6.3
Na (mmol/L)	148 (140–161)	37	21.6	0	140–153
K (mmol/L)	4.7 (3.0–6.5)	37	5.4	10.8	3.8–5.5
Cl (mmol/L)	111 (98–125)	37	24.3	8.1	107–115
Coagulation panel					
PT seconds	7.9 (6.4–11.1)	37	24.3	0	5.0–8.5
PTT seconds	12.9 (9.7–16.4)	37	13.5	0	9.0–14.0

RBC, red blood cell; WBC, white blood cell; PLR, platelet to lymphocyte ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; Chol, cholesterol; BUN, blood urea nitrogen; P, phosphorus; Na, sodium; K, potassium; Cl, chloride; PT, prothrombin time; PPT, partial thromboplastin time.
*Indicates a p value < 0.05 based on Cox proportional hazard analysis.

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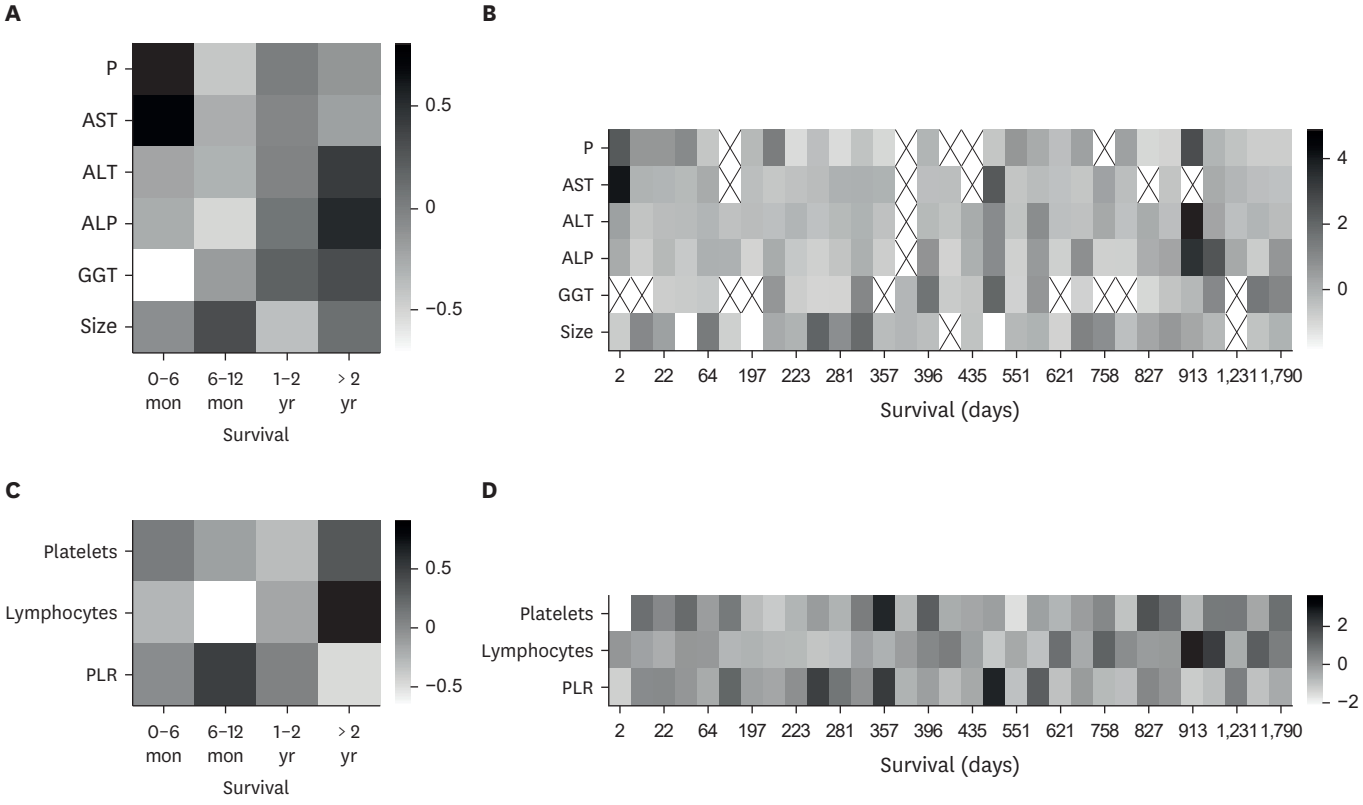

Fig. 1. Heat map of phosphorous and liver enzymes from dogs with HCC by survival. (A) Heat map depicts P, AST, ALT, ALP, and GGT sorted by survival interval (0–6 mon, n = 6; 6–12 mon, n = 8; 1–2 yr, n = 8, > 2 yr, n = 9). (B) Phosphorous and liver enzyme values of individual dogs sorted by survival time (X = no value). (C) Heat map depicts platelet, lymphocyte, and PLR sorted by survival interval (0–6 mon, n = 6; 6–12 mon, n = 8; 1–2 yr, n = 8, > 2 yr, n = 9). (D) Heat map of platelet, lymphocyte, and PLR of individual dogs sorted by survival time (X = no value).
P, phosphorus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; PLR, platelet to lymphocyte ratio.

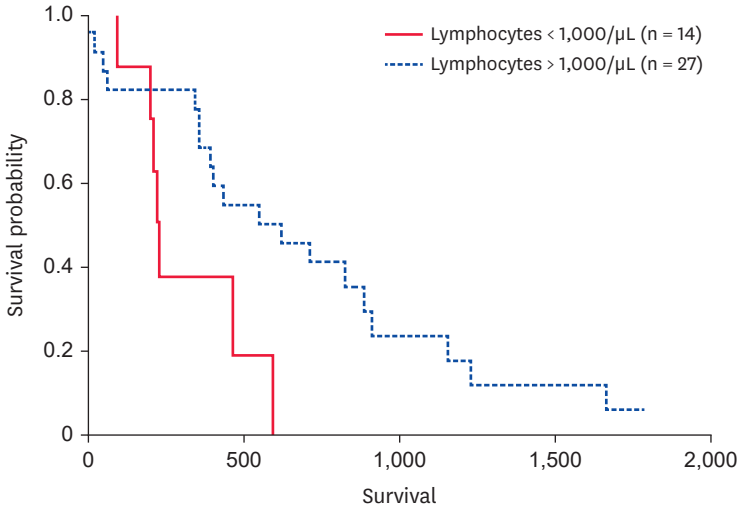

Fig. 2. HCC survival analysis sorted by lymphocyte count. Kaplan-Meier curves of dogs with HCC grouped by lymphocyte count greater than or less than 1,000 lymphocytes per microliter ($p = 0.024$).
HCC, hepatocellular carcinoma.

Platelet: lymphocyte

ROC curve analysis was applied to measure the diagnostic accuracy of PLR as a biomarker. PLR was not a significant predictive factor ($p = 0.15$). Using a binary survival value of less than or greater than 365 d, a PLR ≤ cutoff of 153.8 was determined ($p = 0.15$). Sensitivity and specificity for this value were 47.1% and 91.7%, respectively, with an area under the curve = 0.657.

Diagnostic imaging

AUS and CT reports were available for 41 (97.6%) and 23 (54.8%) patients, respectively. All patients that had CT also had an AUS. A tumor was identified in 35 (85.4%) patients via AUS. The reported diameter of the mass in centimeters (cm) was measured by imaging software electronic calipers was recorded in 33 (94.3%) patients. Four dogs had greater than 1 mass (11.4%). The largest dimension of the neoplasm ranged from 2.1 to 15 cm with a median of 6.0 cm. Twenty-seven patients (81.8%) had a tumor greater than 5 cm. A hepatic mass(es) were identified in 22 (95.7%) dogs via CT. One dog had a generalized nodular appearance without a distinct tumor. The diameter of the tumor was recorded for 19 (86.4%) patients. The location of the tumor was recorded in 21 (95.5%) patients. Six patients had more than 1 mass (27.3%). Using the largest recorded dimension, the median size was 7.7 cm (range: 3.5–15 cm). Fifteen (78.9%) patients had a tumor larger than 5 cm. Tumor size did not correlate with survival in this cohort.

Cytology

Results of cytology acquired by fine-needle aspirate of the tumor were available for 32 of 42 patients. Hepatocellular neoplasia was confirmed cytologically in 21 patients (65.6%). The sensitivity of correctly diagnosing HCC on cytology was 65.6% with a false negative rate of 34.4%.

Variables associated with survival

The location of the neoplasm was significantly correlated with survival ($p < 0.001$). Dogs with HCC in the left lateral liver had the longest survival (MST = 827 d). Dogs that underwent partial or complete surgical resection had a significantly higher MST (462 d) than dogs that did not (MST = 35.5 d). Yet, subsequent analysis determined that tumor location and surgical resection were not independent variables ($p = 0.0267$). Patients with a right-sided mass were less likely to undergo surgery.

DISCUSSION

This work corroborated findings reported by other retrospective studies on canine HCC, including improved survival with surgical resection, the influence of tumor location on survival, and the moderate sensitivity of cytology as a diagnostic test for HCC [1,3,9]. The primary objective of this study was to determine whether PLR could be used to predict survival outcomes in dogs with HCC because thrombocytosis with concurrent lymphopenia portend a shortened survival time in human HCC. Although PLR was not significantly correlated with survival in this small cohort of dogs with HCC, lymphopenia independently predicted a reduced probability of survival.

The scientific theory behind the correlation between lymphopenia and prognosis is thought to reflect changes in the cancer-induced inflammatory response both systemically and in the tumor microenvironment. The patient immune response can attenuate neoplastic progression by means of tumor infiltrative lymphocytes, which promote regulated cell death,

innate immune clearance, and anti-neoplastic response [10-13]. Although controversy exists surrounding the benefit and detriment of lymphocyte subtypes, the peripheral lymphocyte count is thought to be inversely related to tumor growth. The results of this study lead to additional questions: 1) Does the peripheral lymphocyte count mirror that of the tumor microenvironment in canine HCC? 2) Which lymphocyte subtypes promote an anti-neoplastic response, and can this be therapeutically modulated? Further investigation of the local and systemic lymphocyte number and subtypes are warranted in dogs with HCC.

This study had several limitations inherent to its retrospective design. Survival outcome was undetermined for 6 dogs. Uniform data were not available for all cases, leaving gaps in the quantitative analyses. The small sample size may have led to a type II error when evaluating PLR. A larger cohort may identify a correlation between PLR and survival outcomes in canine HCC.

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