Hepatosplenic lymphoma in a dog

Yvonne Wikander DVM[1,2], Susan Nelson DVM[3,4], Brandy Kastl DVM[1,2], Jonathon Sago DVM[1,2], Sarah Schneider DVM DACVP[1,2], William Vernau BSc, BVMS, DVSc, PhD[5], Diana Schwartz DVM, DACVP[1,2],

[1]Department of Diagnostic Medicine/Pathobiology, Kansas State University College of Veterinary Medicine, Manhattan, KS

[2]Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS

[3]Department of Clinical Sciences, Kansas State University College of Veterinary Medicine

[4]Veterinary Health Center, Kansas State University, Manhattan, KS

[5]Department of Clinical Pathology, Microbiology & Immunology, University of California, Davis, CA

Phone: +1-503-858-7302

E-mail: ywikander@vet.k-state.edu

SIGNALMENT
10-year-old female spayed Border collie crossbred dog

HISTORY
Wilma presented to the Pet Health Center at Kansas State University for intermittent lethargy, cranial abdominal discomfort, and decreased appetite. Medications include prednisone every other day for previously diagnosed Systemic Lupus Erythematous and atypical Addison's disease.

CLINICAL FINDINGS
Physical examination was largely unremarkable except for mild abdominal distention and discomfort on palpation. CBC, serum biochemistry panel, urinalysis, and bile acids were performed (results below). Abdominal radiographs and ultrasound demonstrated mild splenomegaly and hepatomegaly. Ultrasound-guided liver and splenic aspirates were obtained for cytology.

LABORATORY findings:

ANALYTE	UNITS	VALUE	REFERENCE INTERVAL
PCV	%	40	41-59
RBC	X 10^9/uL	5.25	5.8-8.2
Hgb	g/dL	13.1	14.1-20.5
MCV	fL	77.4	64-76
MCHC	g/dL	32.4	33-36
RDW	%	14.4	11.4-13.7
RETICULOCYTE	M/uL	0.09	0.01-0.12
RBC NOTES: Occasional polychromasia, occasional keratocytes, rare Howell-Jolly bodies			

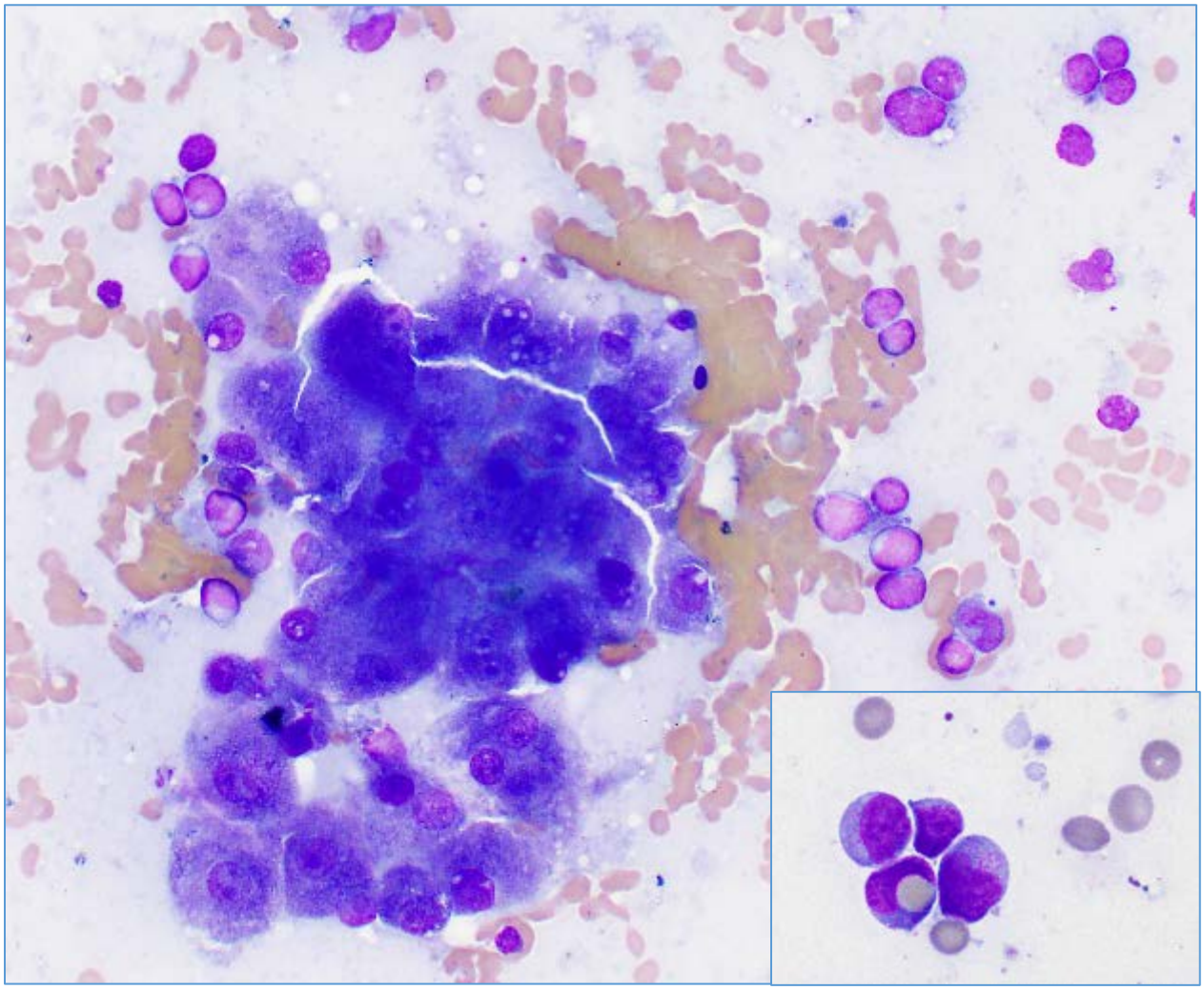
WBC	X 10^3/uL	10.0	4.3-13.6
NEUTROPHILS	X 10^3/uL	8.6	2.5-9.3
BANDS	X 10^3/uL	0	0-0.1
LYMPHOCYTES	X 10^3/uL	0.4	0.8-4.3
MONOCYTES	X 10^3/uL	0.8	0.1-0.9
EOSINOPHILS	X 10^3/uL	0.2	0-1.5
BASOPHILS	X 10^3/uL	0	0-0.1
PLT	X 10^3/uL	165	130-370
PLT NOTES: Clumps noted			
TP (refractometry)	g/dL	5.5	6.3-8.0

ANALYTE	UNITS	VALUE	REFERENCE INTERVAL
TOTAL PROTEIN	g/dL	4.4	5.3-6.9
ALBUMIN	g/dL	2.7	3.2-4.2
GLOBULIN	g/dL	1.7	1.8-3.0
UN	mg/dL	12	8-29
CREATININE	mg/dL	0.7	0.6-1.4
GLUCOSE	mg/dL	101	70-120
CHOLESTEROL	mg/dL	383	140-390
BILIRUBIN, TOTAL	mg/dL	0.7	0-0.2
ALT	U/L	923	20-144
ALP	U/L	621	10-130
CK	U/L	205	54-226
CALCUM	mg/dL	8.5	9.5-11.2
PHOSPHORUS	mg/dL	4.5	2.2-6.1
SODIUM	mmol/L	150	144-151
CHLORIDE	mmol/L	117	106-117
POTASSIUM	mmol/L	4.7	3.7-5.0
HCO_3^-	mmol/L	22	18-24
ANION GAP	mmol/L	17	18-27
SERUM NOTES: Icterus			
PT	Seconds	9.7	7-10
PTT	Seconds	14.7	9-15

ADDITIONAL TESTS

Bile Acids – Pre-prandial 11 µmol/L (5-34); Post-prandial 48 µmol/L (No RI)

LIVER ASPIRATE (Wright-Giemsa, 50x; inset 100x)

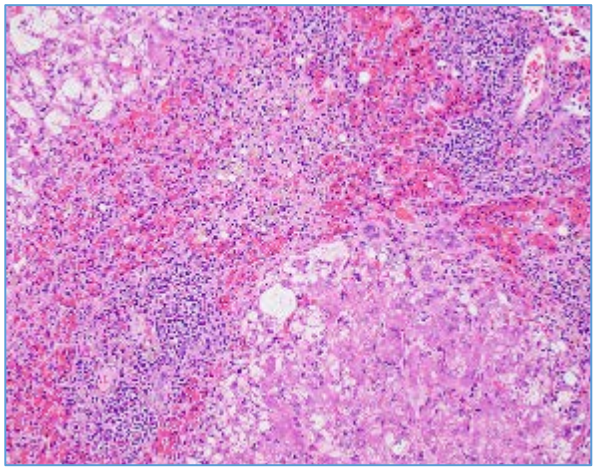


A similar infiltrate of atypical lymphocytes was seen on the splenic aspirate.

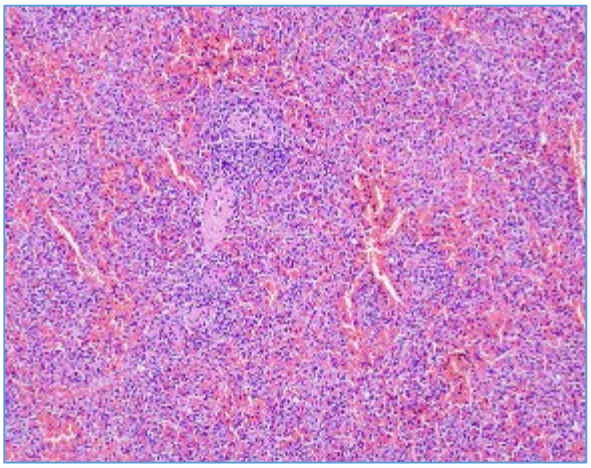
OUTCOME

The owner elected for palliative care and the patient was euthanized within a week due to her declining condition. The body was submitted for necropsy.

HEPATIC HISTOPATHOLOGY (H & E, 20x)



SPLENIC HISTOPATHOLOGY (H & E, 20x)



QUESTIONS:

1. Based on the cytomorphology and reported tissue distribution of neoplastic lymphocytes in this case, what immunophenotype(s) should be considered for these neoplastic cells?

2. What are reported primary locations for lymphoma of granular lymphocyte type in veterinary species?

INTERPRETATION/DIAGNOSIS

Lymphoma, granular lymphocyte type, compatible with Hepatosplenic lymphoma

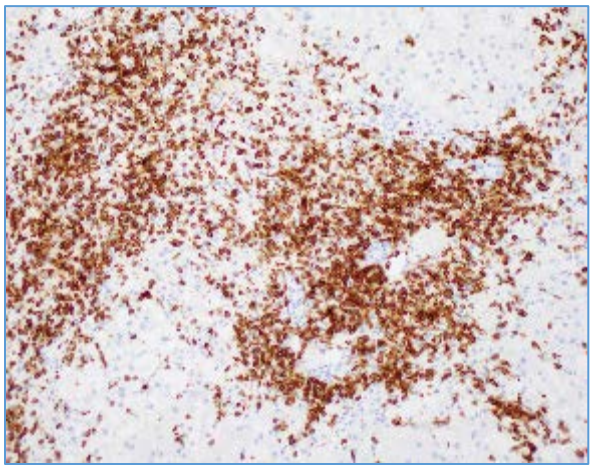
Laboratory findings indicate a mild, non-regenerative anemia; mild panhypoproteinemia (predominantly albumin); hypocalcemia (secondary to hypoalbuminemia); mild hepatocellular injury (ALT); and intrahepatic cholestasis and/or steroid hepatopathy (Bili, ALP).

An expanded population of intermediate lymphocytes was observed in the splenic and hepatic aspirates. These lymphocytes had a moderate amount of pale blue cytoplasm with fine, often packeted, magenta granules. Nuclei were indented to oval, central to eccentric (approximately 1.5-2 RBC diameter) with finely stippled chromatin and largely inapparent nucleoli.

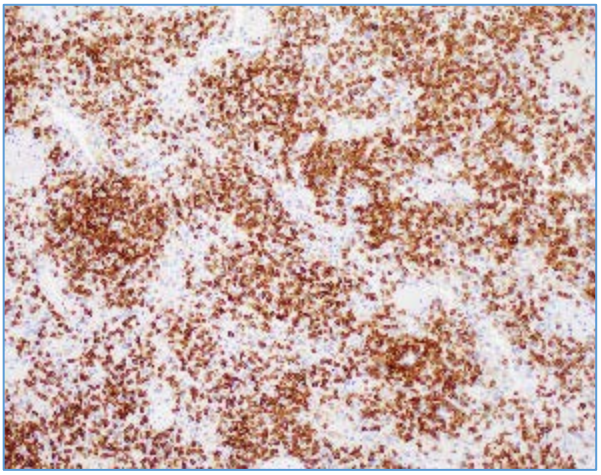
Histology confirmed the presence of numerous intermediate-sized lymphocytes within the spleen, liver, and abdominal lymph nodes. In the spleen, sheets and cords of lymphocytes were filling and expanding the sinusoids. In the liver, lymphocytes were primarily observed in the periportal regions. Many erythrophagocytic macrophages were also present throughout the liver and spleen.

Sections of liver and spleen were incubated with antibodies against CD3 and CD20. The lymphocytes displayed strong, diffuse cytoplasmic and membranous CD3 positivity, and did not express CD20. To confirm these lymphocytes were neoplastic, PCR for Antigen Receptor Rearrangement through Colorado State University (PARR) was performed and a clonal T-cell receptor population was identified.

HEPATIC HISTOPATHOLOGY (CD3, 20x)

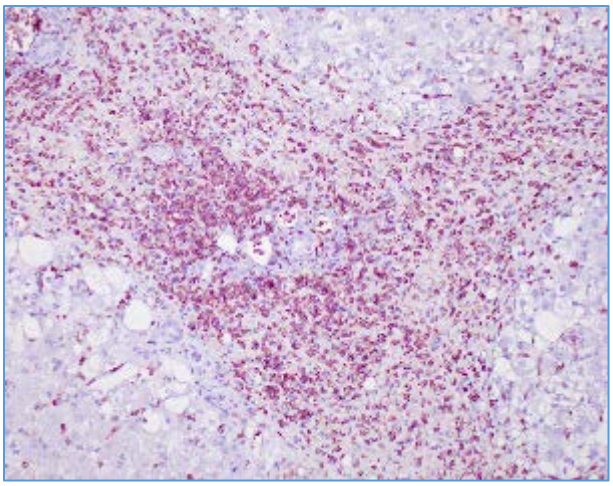


SPLENIC HISTOPATHOLOGY (CD3, 20x)

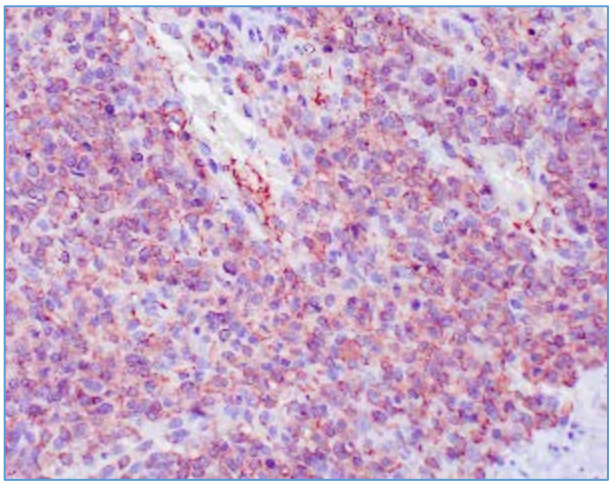


Sections of liver and spleen were submitted to University of California Davis where they were incubated with antibodies against CD11d. The lymphocytes displayed strong, diffuse cytoplasmic and membranous CD11d positivity supporting the diagnosis of hepatosplenic lymphoma.

HEPATIC HISTOPATHOLOGY (CD11d, 20x)



SPLENIC HISTOPATHOLOGY (CD11d, 50x)



ANSWERS TO QUESTIONS

The cytologic finding of a lymphoma of granular lymphocyte type suggests a T-cell or Natural Killer (NK) cell origin.[1] **[Q1]** Morphologically, NK and granular T-cells are indistinguishable.[1] NK cells diverge from precursor T-cells very early in lymphocyte development and, while they express general leukocyte markers (CD18, CD45), they usually do not express typical lymphocyte lineage associated markers, with the exception of CD8α.[1] T-cell receptors are heterodimers, which can be composed of either αβ or γδ subunits. Lymphocyte precursors that continue developing into granulated T-cells will differentiate into either $CD4^{-}CD8^{-}$ γδ TCR^{+}, $CD8^{+}$ γδ TCR^{+}, or $CD8^{+}$ αβ TCR^{+} cells.[1] Those T-cells originating from within the splenic or bone marrow red pulp, such as hepatosplenic lymphoma, will also demonstrate $CD11d^{+}$.[1] **[Q2]** Neoplasia arising from granular lymphocytes can be seen in a variety of tissues and are most commonly identified in the gastrointestinal tract (cats, horses, dogs), spleen (dogs), liver (dogs), thymus (dogs), nasal mucosa (cats), and skin (dogs) of veterinary species.

T-cells with γδ-TCR tend to demonstrate epitheliotropism, concentrating at mucous membranes and within the splenic red pulp.[1,6] Few of these cells are found circulating in peripheral blood; domesticated ruminants and porcine species being exceptions. Although their exact functions are not yet known, it is thought that they bridge the innate and adaptive immune systems.

DISCUSSION

Lymphoma is a heterogeneous group of diseases that, in humans, are classified using the World Health Organization (WHO) scheme based on clinical, morphologic, immunophenotypic, and genetic criteria. This classification scheme continues to undergo modification to improve the diagnosis and treatment of patients, and to predict prognosis. Many canine lymphoma classification schemes exist including Rappaport, Dorfman, Lukes & Collins, Kiel, British Lymphoma, Working Formulation, Revised European-American Lymphoma (REAL), and WHO systems.[2–6] Although none of these classification systems has been uniformly adopted, a common approach based on a modification of the WHO classification scheme for human lymphomas has been proposed. This scheme is currently divided into seven categories, with an initial distinction between Hodgkin-like vs Non-Hodgkin forms. Subsequently, non-Hodgkin lymphomas are further subdivided by lymphocyte phenotype (B-cell vs T-cell), and degree of

maturation (precursor vs mature forms).[4,7] These different types of lymphoma are associated with different biologic behavior or grade (e.g. low vs high).

Many different types of canine lymphoma can involve the spleen and liver. As such, clinical presentation, cytomorphologic findings, histologic distribution, and immunophenotype are necessary to properly classify lymphoma, and predict its biological behavior. Two high-grade lymphomas, hepatosplenic (HS) lymphoma and hepatocytotropic (HC) lymphoma, can both arise from granular lymphocytes and involve the liver.[6] Although hematologic and biochemical abnormalities may overlap, HS lymphoma is typified by regenerative anemia, thrombocytopenia, and hypoproteinemia, while HC lymphoma is associated with a marked cholestasis.[6]

Both HS and HC lymphoma are thought to originate from cytotoxic T-cells, predominantly γδ T-cells.[6] Histologically, HS lymphoma tends to concentrate neoplastic lymphocytes in the hepatic and splenic sinusoids, while HC lymphoma lymphocytes localize to the hepatic cords, resulting in marked cholestasis.[6] Regardless, both HS and HC lymphomas have an aggressive biologic behavior with rapid progression of symptoms and euthanasia or death within one month of initial diagnosis.[6,8]

In our case, the immunophenotype and cytomorphology of the lymphocytes was most compatible with HS lymphoma, even though the histologic distribution in the liver was not typical. This case illustrates the importance of utilizing and synthesizing multiple different diagnostic modalities to best characterize a lymphoma and predict its biologic behavior.

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