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CASE INFORMATION:

Lilly, a 4 ½-year-old female spayed Old English Mastiff, presented to the Purdue University Veterinary Teaching Hospital emergency service on referral from her primary veterinarian (rDVM). Thrombocytopenia, elevated liver enzymes (ALT: 1294, RI: 3-69; ALP: 1214, RI: 20-157; GGT: 26, RI: 5-16), total bilirubin (T. bili: 5.20, RI: 0.10-0.80), and increased respiratory rate were the primary abnormalities identified by the rDVM. Upon initial examination, her temperature was elevated (103.4°F), and the popliteal, prescapular, and facial lymph nodes were enlarged. A CT scan revealed hepatosplenomegaly, mild sternal lymphadenopathy, and a small amount of peritoneal effusion.

LABORATORY DATA:

HEMOGRAM RESULTS

TEST	RESULT 4/25/19	RESULT 4/26/19	REFERENCE INTERVAL	UNITS
HCT	37.1	**27.1**	37.0-55.0%	%
RBC	**5.27**	**2.84**	5.5-8.5	M/µL
Hb	12.3	**9.0**	12.0-18.0	g/dL
MCV	70.4	70.5	60.0-75.0	fl
MCHC	33.3	33.2	32.0-36.0	g/dL
RETIC	34.4	36.3	<100	K/µL
Total protein	**5.5**	**4.6**	6.8-8.0	g/dL
WBC	**4.6**	**2.7**	6.0-17.0	K/µL
Segmented Neutrophils	**2.7**	**1.4**	3.0-12.0	K/µL
Band Neutrophils	0.09	0.22	0.00-0.30	K/µL
Lymphocytes	**0.3**	**0.2**	1.0-5.0	K/µL
Monocytes	0.32	**0.08**	0.15-1.35	K/µL
Large granular lymphocytes**	**1.24**	**0.76**	0.00-0.00	K/µL
Platelets	**21**	**17**	200-500	K/µL

**Figure 1

SERUM CHEMISTRY RESULTS

TEST	RESULT 4/26/19	REFERENCE INTERVAL	UNITS
Glucose	**61**	67-132	Mg/dL
Blood Urea Nitrogen	29	7-32	Mg/dL
Creatinine	1.00	0.50-1.50	Mg/dL
Phosphorus	4.9	2.2-7.9	Mg/dL
Calcium	**7.9**	9.7-12.3	Mg/dL

Sodium	**135**	138-148	Mmol/L
Potassium	4.1	3.5-5.0	Mmol/L
Chloride	107	105-117	Mmol/L
Carbon Dioxide	15	13-24	Mmol/L
Anion Gap	17.1	9.0-18.0	Mmol/L
Total Protein	**4.3**	4.8-6.9	g/dL
Albumin	2.4	1.7-3.8	g/dL
Globulin	1.9	1.7-3.8	g/dL
A/G ratio	1.3	0.8-1.9	--
ALT	**1294**	3-69	IU/L
Alkaline Phosphatase	**1214**	20-157	IU/L
GGT	**26**	5-16	IU/L
Total bilirubin	**5.20**	0.10-0.80	Mg/dL
Cholesterol	**105**	125-301	Mg/dL
Amylase	439	378-1033	IU/L
Lipase	178	104-1753	IU/L
Fibrinogen	144	69-289	Mg/dl

ADDITIONAL TESTING:

TEST	RESULT 4/26/19	REFERENCE INTERVAL	UNITS
Ammonia	<8.7	1.0-46.0	µmol/L
4Dx***	Negative	Negative	Negative

*** *A. Phagocytophilum/ A. platys, B. burgdorferi, D. immitis, E. canis/E. ewingii*

CYTOLOGIC IMAGES:

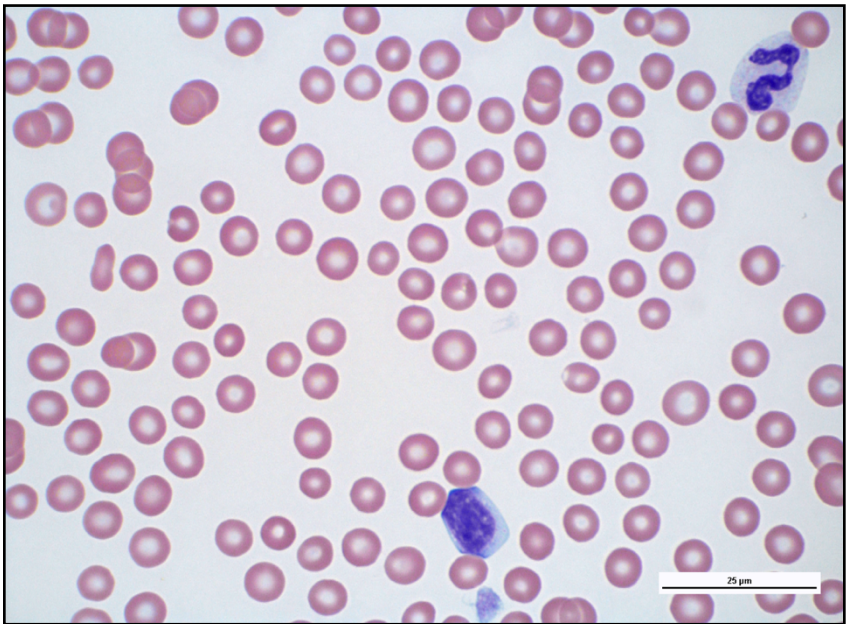

FIGURE 1. Blood Smear, 100x. Modified Wright's Stain. Large granular lymphocyte: this photomicrograph represents a mononuclear cell with a moderate amount of pale blue cytoplasm containing small, dusty pink granules and a moderately clumped chromatin pattern.

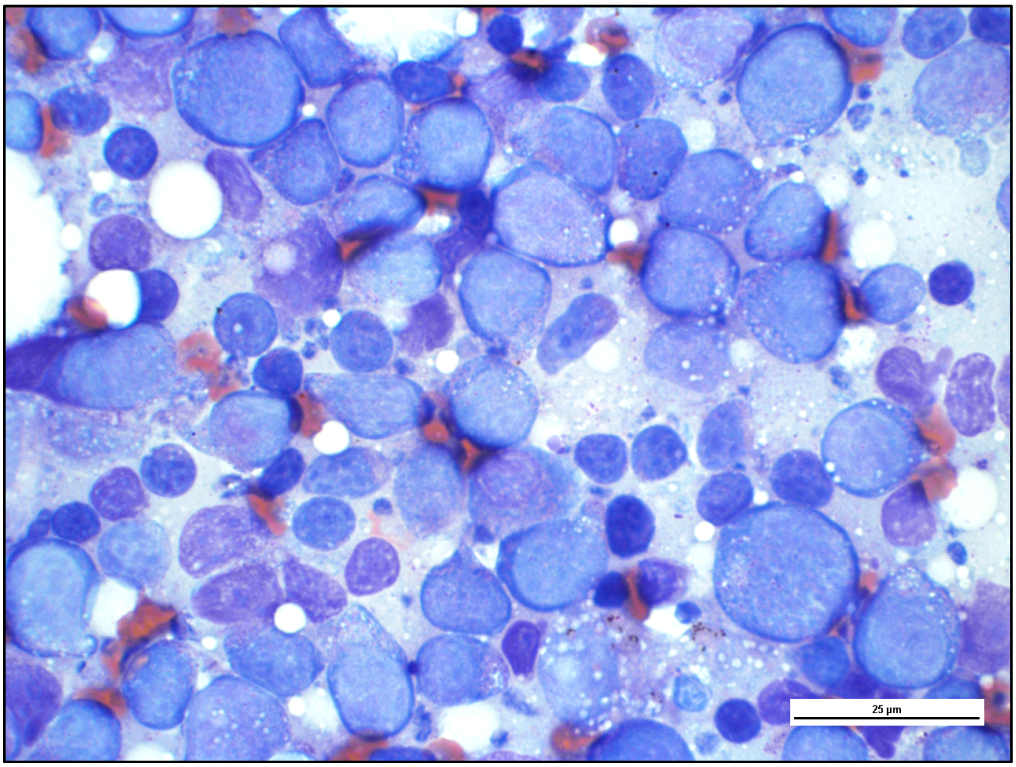

FIGURE 2. Lymph node aspirate, 100X. Modified Wright's Stain. The lymphoid population consisted of high numbers of intermediate to large lymphocytes. The lymphoid cells had small to moderate amounts of basophilic cytoplasm that often contained a dusting of pink granules. Their nuclei had a moderately clumped to finely stippled chromatin pattern and occasionally contained one to two medium-sized prominent nucleoli.

ADDITIONAL FINDINGS: A bone marrow aspirate and core biopsy were evaluated. These preparations were highly cellular and of excellent diagnostic quality. Both the aspirate and core displayed moderate myeloid and erythroid hypoplasia along with ineffective thrombopoiesis. On the bone marrow core, there was evidence of bone remodeling and mild osteosclerosis. In addition, a prominent population (~15% of all nucleated cells) were large granular lymphocytes (LGLs), morphologically similar to the LGLs from the peripheral blood and lymph nodes (Figure 3). A histiocytic population was also present; these often displayed erythroid phagocytosis, and less frequently, myeloid phagocytosis (Figure 3 and Figure 4).

Immunohistochemistry was evaluated on the bone marrow core, the round cells were strongly positive for CD18 and CD3, and there was scattered immunoreactivity for CD11d (Figure 5). Further evaluation via immunocytochemistry revealed only scattered lymphocytes positive for either CD4 or CD8α.

PCR FOR ANTIGEN RECEPTOR REARRANGEMENT (PARR):
Bone marrow was submitted for PARR analysis and displayed a clonally rearranged of the T cell receptor gamma gene.

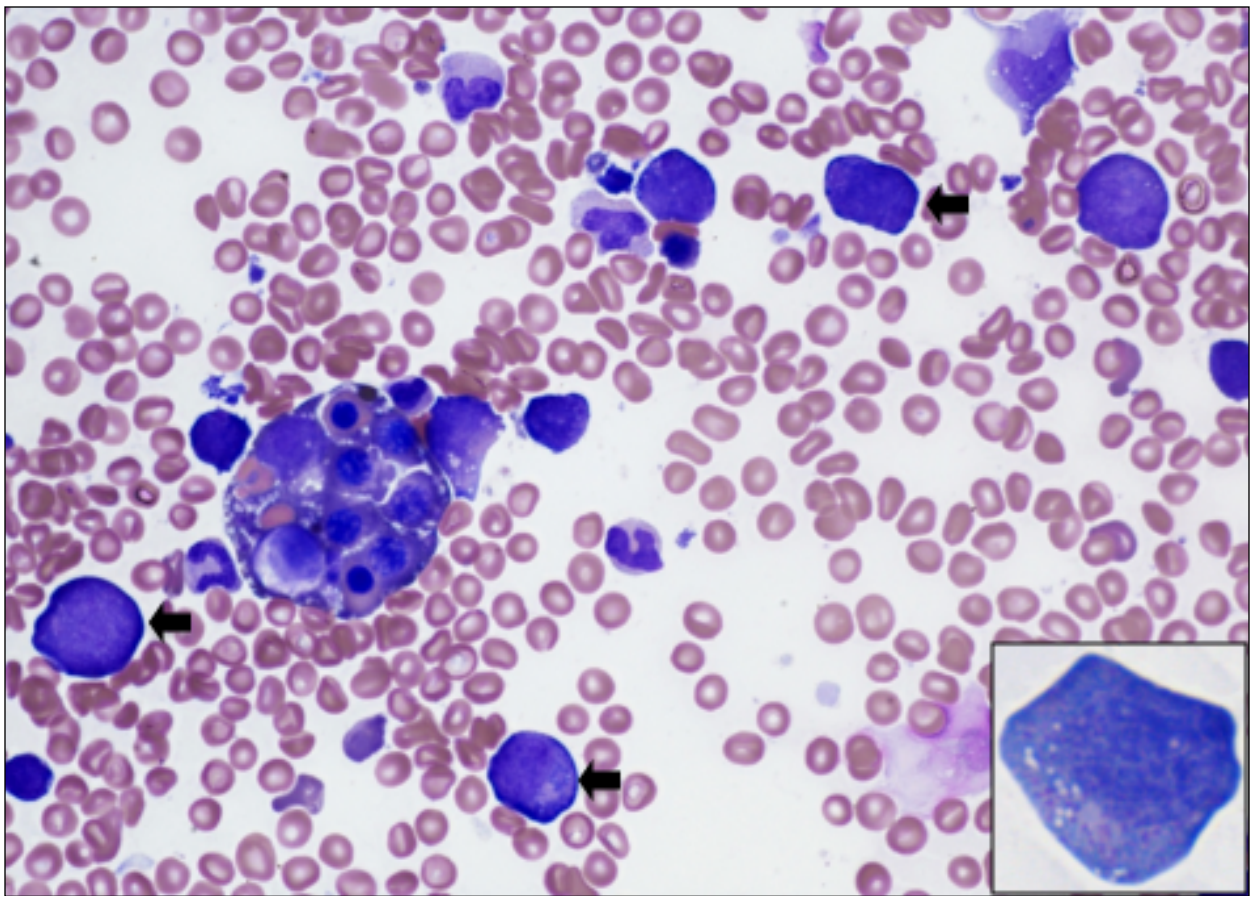

Figure 3. Bone marrow aspirate, 100x. Modified Wright's stain. Several large granular lymphocytes (arrows) and a macrophage displaying phagocytosis of erythroid precursor cells, mature erythrocytes, and a single leukocyte are noted. **Inset:** An enlarged image of the large granular lymphocyte to highlight the granularity of the cell.

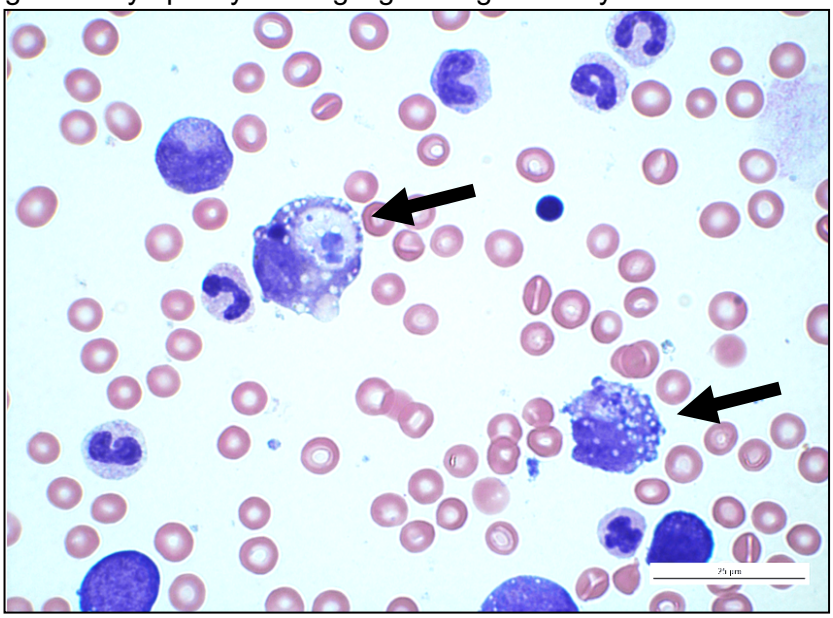

Figure 4. Bone marrow aspirate, 100x. Modified Wright's stain. Low numbers of histiocytes displaying phagocytosis are seen (arrows).

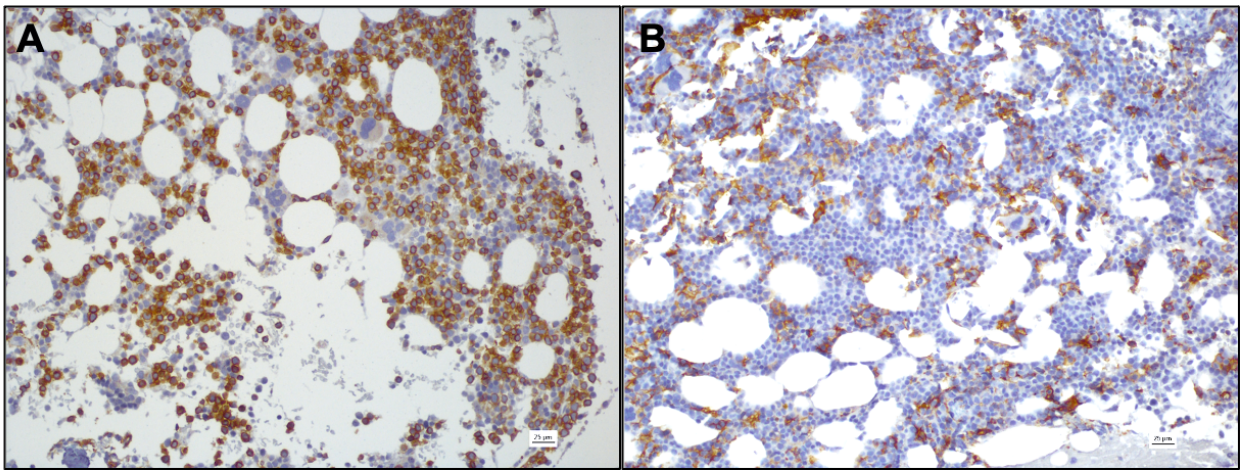

Figure 5. Bone marrow core, 20X. A. IHC for CD3 showing marked immunoreactivity by a population of lymphocytes widely spread throughout the bone marrow space. **B.** IHC for CD11d showing scattered positive cells.

QUESTIONS:

1. What is the cause of the prominent phagocytosis of both myeloid and erythroid precursor cells in this case?

2. Large granular lymphocytes can belong to what distinct cell types, and how might this information direct additional testing to characterize the neoplastic LGLs in this case?

INTERPRETATION/DIAGNOSIS: Large granular lymphoma with associated hemophagocytic syndrome

ANSWERS TO QUESTIONS:

1. This is likely due to macrophage activation syndrome, a form of hemophagocytic syndrome (HPS), which can happen secondary to various diseases, including neoplasia, autoimmune disease, and infectious disease. The underlying disease/disorder can lead to immune dysregulation and cytokine storm, resulting in abnormal cytokine production levels, such as IFN-ɣ, IL-6, IL-12, IL-18, and TNF-α and subsequent indiscriminate destruction of host cells by activated macrophages[1-6].

2. LGL lymphomas may be cytotoxic T cells, expressing either TCRαβ or TCRɣδ, or natural killer cells.

    - In this case, the strong positivity for CD3 indicates that the neoplastic lymphocytes are T lymphocytes.
    - The majority of the neoplastic lymphocytes, in this case, were negative for CD4. Thus the neoplastic cells are not T-helper cells, but they were also negative for CD8αα. CD8αβ may be evaluated to confirm this is not a typical cytotoxic T cell phenotype. While T-LGL malignant cells are usually $CD3^+$, $CD4^-$, and $CD8^+$, many reports in the human literature describe patients with $CD4^+$, $CD8^-$ LGL leukemia, dual positive $CD4^+/CD8^+$, and dual negative $CD4^-/CD8^-$ cases[5].
    - In this case, the expression of TCRɣδ by the malignant cells would suggest a red pulp splenic or epithelial origin of the neoplastic LGLs. However, the results of this marker were inconclusive. The absence of CD11d expression by the neoplastic cells indicates these cells are not of splenic red pulp origin.
    - No specific markers for NK cells in dogs are currently available; however, recent studies suggest that $CD3^-$ $NCR1^+$ (NKp46, CD335) likely represent canine NK cells. Although NCR1 was not evaluated in this case, the strong expression of CD3 by the neoplastic population suggests these cells are not of the NK phenotype[3].

    Summary: The neoplastic cells were $CD3^+$, $CD4^-$, $CD8^-$, had an LGL morphology and were associated with HPS in this case.

OUTCOME/FOLLOW-UP:

The day after the presentation to PUVTH, Lilly went into cardiac arrest, and cardiopulmonary resuscitation was unsuccessful. The owners declined necropsy.

DISCUSSION:

Hemophagocytic syndrome (HPS) is a rare disorder characterized by a dysregulated immune response, which results in the uncontrolled activation of macrophages and indiscriminate destruction of host cells[1-6]. Phagocytosis of hematopoietic precursors in the bone marrow and cytopenias in peripheral blood are salient features that help to define HPS. This condition has been described in dogs, cats, and humans[8]. In humans, the syndrome has been further classified as primary HPS, associated with genetic mutations and development during childhood, and secondary HPS, associated with an underlying disease process[1,2,4,6–8]. While this disease affects one in 800,000 people per year, a low number (5-10%) of patients have no underlying etiology and are classified as idiopathic[4]. The inherited form of the disease in humans has been associated with homozygous mutations of genes involving CD8+ T-cell and

NK-cell mediated immunity[2]. Secondary HPS is most often found in patients with hematologic malignancies, autoimmune diseases, or iatrogenic immunosuppression[2]. T-cell or NK/T-cell lymphoma (lymphoma-associated HPS: LAHS) is the most common underlying condition of malignancy-associated HPS in humans.[6] In canine patients, HPS has been associated with viral and bacterial infections, autoimmune disorders, and lymphoma (LAHS)[6]. Infectious agents associated with HPS include parvovirus, *Salmonella* spp.*, E. Coli, Blastomyces* sp.*,* and *Borrelia* sp.[8]*.*

Diagnosing HPS can be challenging as many diseases may present with cytopenia. Generally, the cytopenias in HPS are thought to result from hemophagocytosis since the bone marrow in these animals tends to be hypercellular[8]. There are no pathognomonic features for the diagnosis of HPS; however, in humans, the diagnosis is based on fulfilling five out of eight clinical and laboratory criteria, which strongly supports the diagnosis of HPS. These criteria include the presence of fever, splenomegaly, peripheral blood cytopenia in more than one cell line, hypertriglyceridemia and/or hypofibrinogenemia, identification of hemophagocytosis (in the bone marrow, spleen, lymph node, or liver), low to absent NK cell activity, elevated ferritin, and elevated soluble CD25 (soluble IL-2 receptor alpha)[4,6]. In dogs, a modified set of criteria has been proposed. This includes the presence of bicytopenia or pancytopenia in the blood and >2% hemophagocytic macrophages in the bone marrow aspirates[6]. To specifically diagnose LAHS, Suwa and collaborators (2018)[4] recommend the following criteria: 1) the presence of lymphoma, 2) bicytopenia or pancytopenia in the blood, and 3) increased hemophagocytosis in the reticuloendothelial organs[6].

In the current case, the patient met four out of the eight recommended criteria for humans: 1) fever, 2) splenomegaly, 3) cytopenias in all three lineages, and 4) hemophagocytosis in the bone marrow with ~3.5% hemophagocytes. This patient was diagnosed with lymphoma. The neoplastic lymphocytes had a large granular appearance (LGLs) and were strongly CD3+ on immunohistochemistry, confirming their T-cell origin. The findings herein are consistent with previously reported cases of canine LAHS, where dogs presented with fever, splenomegaly, and, in all cases, a T-cell lymphoma, specifically characterized as LGLs, was diagnosed.[1,6].

LAHS is often a life-threatening disease. The uncontrolled activation of the immune system can result in an overwhelming cytokine storm and severe inflammation. Ultimately, multi-system end-organ damage caused by massive inflammation may lead to fatality[2]. A two-tiered approach is suggested for treating human patients; eight weeks of induction treatment with dexamethasone and etposide, followed by tapering of the steroids according to the patient's response. However, the prognosis is still poor[4]. Dogs with LAHS also have poor survival times. As in most human patients with LAHS, the dog herein had cholangiohepatitis and died the day following the bone marrow procedure.

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