



WEDNESDAY SLIDE CONFERENCE 2018-2019

Conference 23

24 April 2019

Conference Moderator:

Amy Durham, BA, MS, VMD, DACVP
Associate Professor, Anatomic Pathology
Faculty Director Comparative Pathology Core
Univ. of Pennsylvania School of Veterinary Medicine

CASE I: 12-324-3 (JPC 4032713).

Signalment: 6-year-old, spayed female, Labrador Retriever cross, dog, *Canis familiaris*

History: The dog presented to the referring veterinarian with a 3-day history of inappetance, vomiting, diarrhea, and lethargy. Radiography revealed an enlarged left kidney. A nephrectomy was performed and the dog recovered uneventfully. The following day the dog became lethargic and then died.

Gross Pathology: The carcass and nephrectomized left kidney were submitted for gross examination. Necropsy examination revealed severe hemoperitoneum, and death was attributed to exsanguination from the surgical excision site. Both kidneys were markedly enlarged and had irregular contours. On section the cortex and medulla were irregularly expanded by discrete to coalescing white patches and streaks. The



Kidney, dog. The cortex contains numerous bulging white streaks. (Photo courtesy of: Diagnostic Services Unit, University of Calgary Veterinary Medicine, Clinical Skills Building, 11877 85 St. NW, Calgary AB T3R 1J3, <http://vet.ucalgary.ca/>)

liver contained approximately a dozen firm white parenchymal nodules up to 1 cm in diameter and had a diffusely accentuated lobular pattern. The mesenteric, renal and thoracic lymph nodes were moderately enlarged and, on section, lacked typical corticomedullary architecture.

Laboratory results: NA

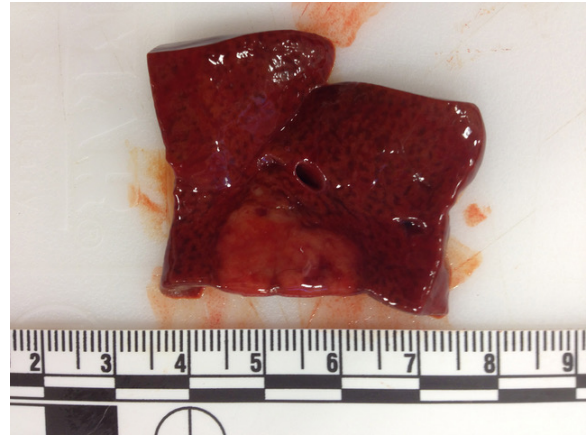
Microscopic Description:

Liver: Two sections of liver are examined. One contains a 9mm diameter nodule (described in the gross pathology section above), and the other is from an unaffected part of the liver.

The nodule is unencapsulated but well circumscribed, slightly infiltrative at its edge and surrounded by a rim of compressed hepatic parenchyma. It consists of sheets of neoplastic cells forming two distinct populations of (a) small round cells and (b) larger, highly pleomorphic cells.

The first cell population makes up approximately 80-90% of the mass. Cells are small (8-15 µm diameter) and round with centrally to eccentrically placed nuclei and a scant to moderate amount of finely granular to homogeneous eosinophilic cytoplasm. Nuclei are round or slightly indented and mildly anisokaryotic with coarsely clumped chromatin and mainly inconspicuous nucleoli. In 10 HPF there are 4 mitotic figures.

The second population of neoplastic cells consists of several hundred highly pleomorphic cells from 15-150 µm in diameter scattered randomly among the smaller cells described above. The most prominent are giant cells with abundant amphophilic to eosinophilic cytoplasm, from 1 to 10 markedly anisokaryotic nuclei, and bizarre nuclear shapes and arrangements. However, numerous forms of these cells intermediate in size or similar in size to the small round cells described above are present. Larger cells often contain variably-sized, brightly eosinophilic, smudged cytoplasmic vacuoles.



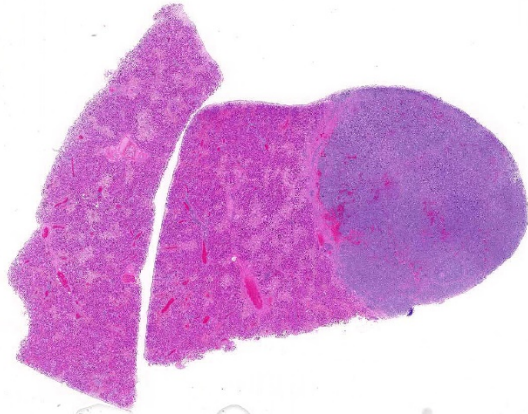
Liver, dog. The liver contains a focal 1cm nodule within the parenchyma. (Photo courtesy of: Diagnostic Services Unit, University of Calgary Veterinary Medicine, Clinical Skills Building, 11877 85 St. NW, Calgary AB T3R 1J3, <http://vet.ucalgary.ca/>)

Throughout remaining (non-nodular) hepatic parenchyma most portal tracts are heavily infiltrated by small round cells similar to those comprising the majority of the nodule described above. Centrilobular sinusoids and central veins are severely congested and centrilobular hepatocytes are atrophied or swollen with foamy cytoplasm (lipid accumulation). Kupffer cells near central veins contain abundant finely granular yellow brown pigment. Multifocally, adjacent centrilobular hepatocytes are outlined by thin, branching green-yellow lines (canalicular cholestasis).

Contributor's Morphologic Diagnoses:

Liver: 1. B cell lymphoma; 2. Megakaryocytic neoplasia

Contributor's Comment: Gross necropsy findings of bilateral irregular renomegaly, multiple pale hepatic nodules, and multifocal abdominal lymphadenomegaly led to a presumptive diagnosis of lymphoma. Histologically, hepatic portal infiltration by sheets of round cells supported this diagnosis. However, in addition to the population of presumed lymphocytes, bizarre giant and blastic cells were seen in solid hepatic



Liver, dog. Subgross examination of a well-demarcated nodular round cell neoplasm. (HE, 4X)

masses, throughout both kidneys and throughout enlarged renal lymph nodes.

IHC was performed on hepatic sections and confirmed a dual population of neoplastic cells in liver masses. The first population of small round cells had strong cytoplasmic immunopositivity for CD20 (fig. 3), a B lymphocyte marker. Cells surrounding portal areas outside the masses were also CD20 positive. The majority of the second population of larger, pleomorphic cells had moderate cytoplasmic immunopositivity for factor VIII-related antigen (fig. 4), supporting a megakaryocytic origin. Scattered among the neoplastic cells were small numbers of CD3-positive T lymphocytes (not shown).

The CD20 IHC results support a diagnosis of B cell lymphoma, a common disease in dogs. However the presence of the additional population of factor VIII-related antigen-positive cells was unusual. Their presence suggested acute megakaryoblastic leukemia (AML M7; AMegL; AML7), an acute myeloid leukemia in which the predominant blast cell is of megakaryocytic lineage. Acute megakaryoblastic leukemia (AML M7) is rare but has been reported in humans, dogs and cats. Among canine acute myeloid

leukemias it is the least common form, and has previously been described in fewer than 20 dogs. It is a rapidly progressive disease involving bone marrow, internal organs, and lymph nodes. Affected dogs exhibit anorexia, progressive weight loss, and occasionally spontaneous epistaxis. Thrombocytopenia and anemia are frequently observed. Neoplastic cells are typically immunopositive for megakaryocytic markers CD61 and factor VIII-related antigen, and the platelet marker platelet glycoprotein IIIA. They may be variably positive for leukocytic markers CD45 and CD18 and the hematopoietic precursor marker CD34, but are negative for lymphocyte, monocyte and granulocyte markers.

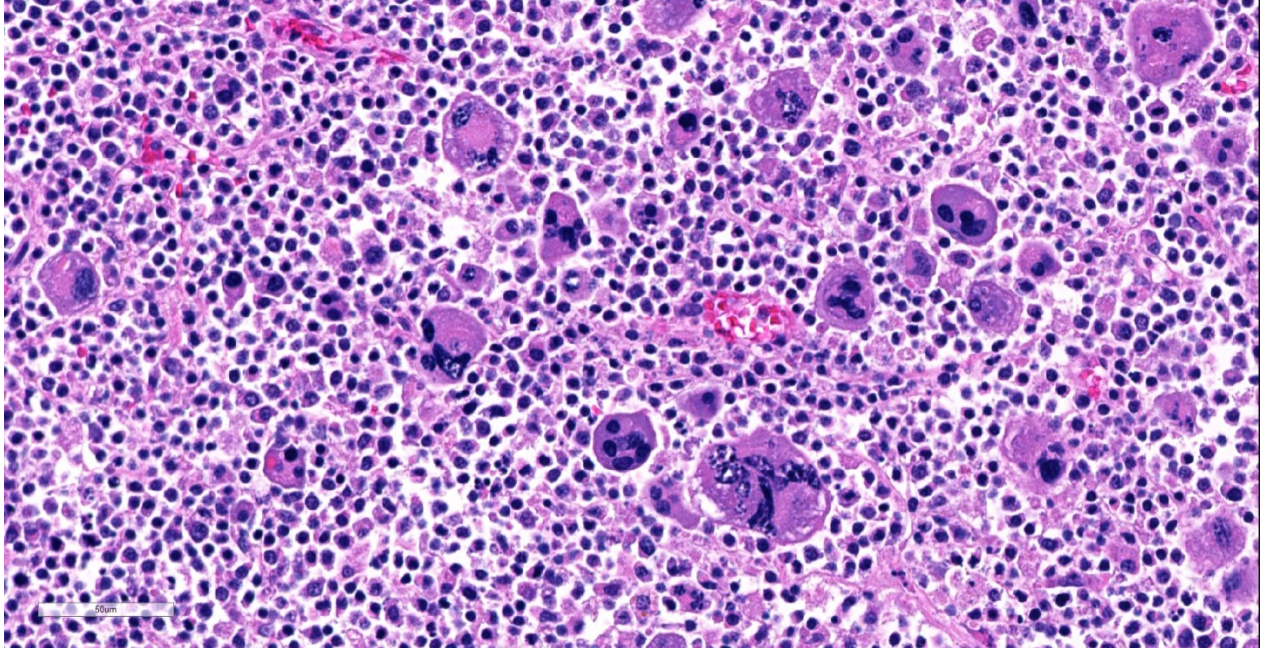
Unfortunately, a potential diagnosis of AML M7 could not be investigated further since neither blood nor bone marrow were available and because financial constraints restricted IHC use. The findings in this case suggest that two forms of hematopoietic neoplasia were present simultaneously and emphasize the need for bone marrow and blood evaluation to achieve a definitive diagnosis.

Contributing Institution:

Diagnostic Services Unit
University of Calgary Veterinary Medicine
Clinical Skills Building
11877 85 St. NW
Calgary AB T3R 1J3
<http://vet.ucalgary.ca/>

JPC Diagnosis: Malignant plasm cell tumor.

JPC Comment: This is an interesting and unique case. The neoplasm in the liver is composed of CD-20 positive B-cells (the immunostaining was confirmed at the JPC) which are the same size or 1.5x the size of an erythrocyte. Neoplastic lymphocytes have a

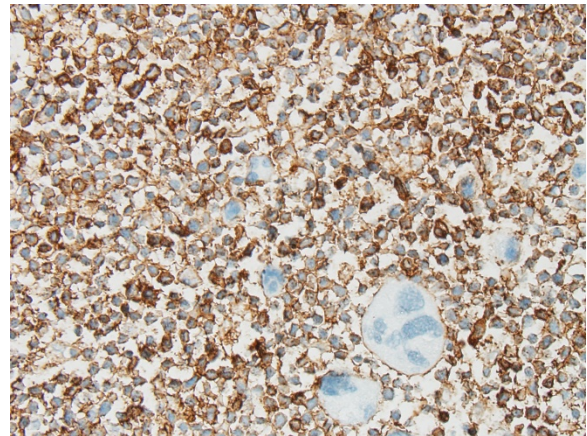


Liver, dog. The neoplasm is composed of two populations – one population of small neoplastic lymphocytes with a low mitotic rate and scattered well-differentiated megakaryocytes. (HE, 400X)

3:1 N/C ratio with a thin rim of non-granulated eosinophilic cytoplasm, and mitotic figures are rare (although preservation is not optimal and made it difficult for attendees to reach consensus on grading this neoplasm.) Nucleoli are not evident in well-preserved cells. Immunostains for CD3, PAX-5 and MUM-1 were negative in this particular population of cells.

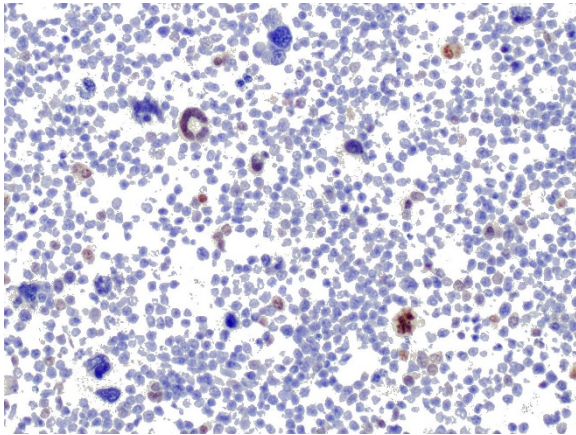
The large pleomorphic cells scattered throughout the neoplasm exhibited strong nuclear staining for MUM-1 run both at the JPC and at UPenn, suggesting that some of the neoplasm cells may be in a late stage of plasma cell differentiation, and coupled with the CD-20 staining of the smaller uninucleate cells that this neoplasm may represent a late stage B cell or malignant plasma cell tumor. This may also explain the non-specific staining of these cells with Factor VIII (as plasma cells are notorious for non-specific immunostaining). This would also help to explain the absence of these atypical cells

outside of the neoplasm (leukemic cells should be in the remainder of the section of liver as well), and the diagnosis of a neoplasm related to myeloma might help to explain the fatal bleeding identified in the clinical history.



Liver, dog. Neoplastic lymphocytes stain strongly immunopositive for CD20. (anti-CD20, 100X). (Photo courtesy of: Diagnostic Services Unit, University of Calgary Veterinary Medicine, Clinical Skills Building, 11877 85 St. NW, Calgary AB T3R 1J3, <http://vet.ucalgary.ca/>)

The small size of the B-cells, lack of prominent nuclei, and low mitotic rate led some conference participants to a diagnosis of mature peripheral B-cell lymphoma /small lymphocytic lymphoma.) B-cell CLL is primarily a neoplasm of the bone marrow which is characterized by marked lymphocytosis (of small mature lymphocytes). The course of these neoplasms is indolent, and tumors may be found in the spleen, liver, and lymph nodes.



Liver, dog. Large pleomorphic cells stain multifocally positive for MUM-1, suggesting plasmacytic differentiation. (anti-MUM-1, 400X)

There is also extensive necrosis and loss of hepatocytes throughout the section in centrilobular and some subcapsular) areas. The precise cause of this change is not evident in the examined slide but is strongly suggestive of hypoxia, perhaps due to terminal shock, anemia (perhaps as a result of marrow infiltration by a neoplasm), or resulting from local vascular impairment as a result of the presence of a neoplasm.

References:

- 1 Colbatzky F, Hermanns W: Acute Megakaryoblastic Leukemia in One Cat and Two Dogs. *Veterinary Pathology* 30: 186-194, 1993
- 2 Comazzi S, Gelain ME, Bonfanti U, Roccabianca P: Acute Megakaryoblastic

Leukemia in Dogs: A Report of Three Cases and Review of the Literature. *Journal of the American Animal Hospital Association* 46: 327-335, 2010

3 Park HM, Doster AR, Tashbaeva RE, Lee YM, Lyoo YS, Lee SJ, Kim HJ, Sur JH: Clinical, histopathological and immunohistochemical findings in a case of megakaryoblastic leukemia in a dog. *Journal of Veterinary Diagnostic Investigation* 18: 287-291, 2006

4 Valli VE, Jacobs RM, Parodi AL, Vernau W, Moore PF: *Histological Classification of Hematopoietic Tumors of Domestic Animals*, 2nd ed. Armed Forces Institute of Pathology in cooperation with the American Registry of Pathology and the World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology, Washington DC, 2002

5 Vernau W, Moore PF: An immunophenotypic study of canine leukemias and preliminary assessment of clonality by polymerase chain reaction. *Veterinary Immunology and Immunopathology* 69: 145-164, 1999

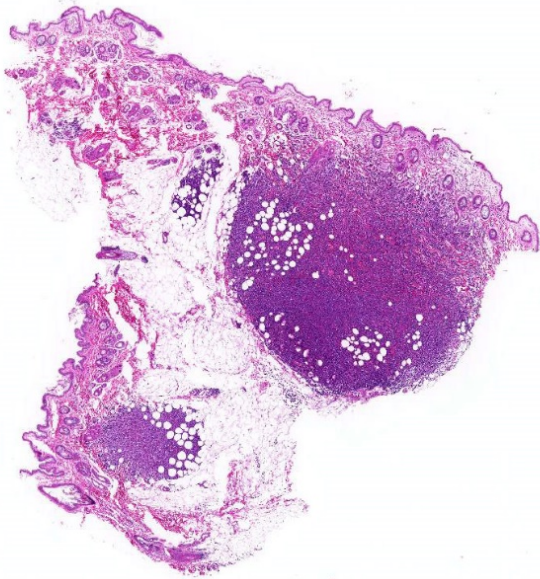
CASE II: 2014910070 (JPC 4066543).

Signalment: 8 year-8 month-old, spayed female, Italian gray hound, dog. (*Canis familiaris*)

History: The dog was presented to the veterinary clinic with skin rashes in inner thigh region. She was treated with antibiotic and antihistamine for a week. A week later, a few millimeters multiple hematomas and papules were observed from inner thigh, abdomen to neck. She was treated with another antibiotic, antiplasmin and steroid, but these lesions were not improved. Three weeks later, a skin in the neck including

papules was biopsied, and submitted to pathological examination.

Gross Pathology: The cut surface of the papule after formalin fixation was brown to dark red.



Haired skin, dog. There are multiple nodular neoplasms within the superficial dermis which extend into the underlying subcutis. (HE, 5X)

Laboratory results: B lymphocyte monoclonality is detected by lymphocyte clonality analysis.

Microscopic Description:

Histologically, a papule is composed of multiple foci. Monomorphic round tumor cells diffusely proliferate in dermis to subcutis of each focus. Tumor cells do not have epitheliotropism. Tumor cells have oval to vesicular nuclei of intermediate-large size and scant to moderate amount of cytoplasm. One small to large nucleolus and finely distributed chromatin contain in the nucleus. Giant and multiple nucleus are occasionally

seen. Mitotic figures are often observed. Mild hemorrhage is seen between tumor cells, and tumor cells frequently engulf red blood cells in the cytoplasm.

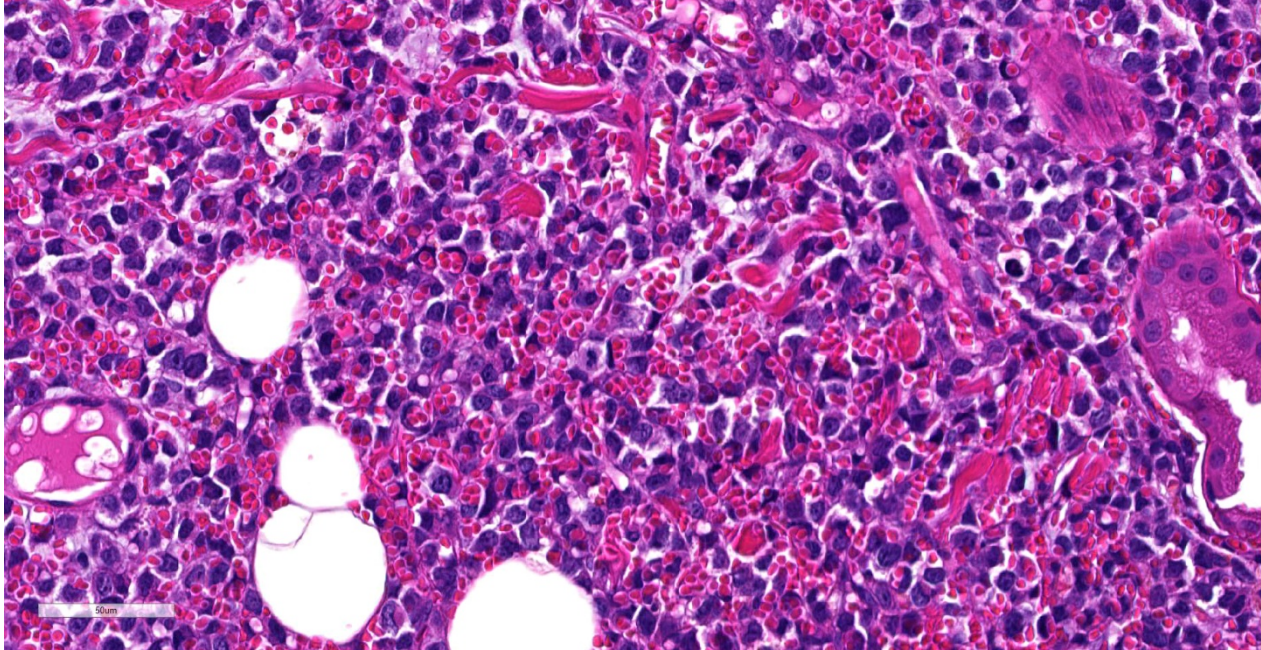
Immunohistochemically, almost all tumor cells are positive for CD20, lambda light chains. Multiple myeloma Oncogene 1, weakly positive for CD79a but negative for CD3, Kappa light chains, and Iba-1.

Ultrastructurally, tumor cells have nucleus with heterochromatin clumped under the nuclear membrane. Cytoplasm contain large amount of rough endoplasmic reticulum, but Golgi complex does not develop. Erythrocytes are taken into tumor cell cytoplasm. Primary and secondary lysosomes are often observed in the cytoplasm.

Contributor's Morphologic Diagnoses: Skin: hemophagocytic cutaneous lymphoma (B cell lymphoma), canine (*Canis familiaris*)

Contributor's Comment: The present case was a round cell tumor with a characteristic of erythrophagocytosis. In animal, erythrophagia was reported most frequently in tumors of histiocytic cell origin^{6,10,14,15,17}. Thus, the primary differential diagnosis was histiocytic sarcoma. However, tumor cells often had scant cytoplasm, and showed no immunohistochemical reactivity for Iba-1 (marker of histiocytic cell).

In animals, erythrophagocytic tumors have been reported several tumors other than histiocytic sarcoma, including angiosarcoma, osteosarcoma, lymphoma, mast cell tumor, multiple myeloma and plasmacytoma^{1-3,12,13,19}.



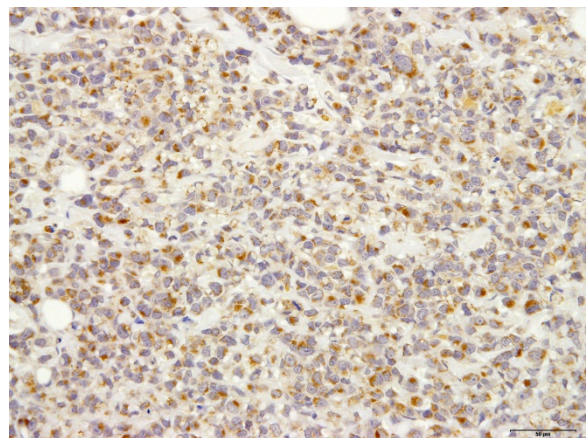
Haired skin, dog. Neoplastic lymphocytes contain one or multiple non-degenerate erythrocytes within their cytoplasm, which often indent or peripheralize the nucleus. Neoplastic cells are four to five times larger than an erythrocyte and have large nuclei with prominent nucleoli. (HE, 400X)

In present case, tumor cells did not have nuclei with a cartwheel appearance and perinuclear halo (clear zone) in cytoplasm - thus, the cellular morphology was not similar to mature plasma cell. However, tumor cells had immunoreaction for CD79a, lambda light chain and MUM-1. MUM-1 was expressed more than 94% of canine plasmacytomas and few canine B cell lymphoma¹⁶. Developed rough endoplasmic reticulum like plasma cell was seen in the cytoplasm by ultrastructural observation. It was suggested tumor cells had partially plasmacytic differentiation.

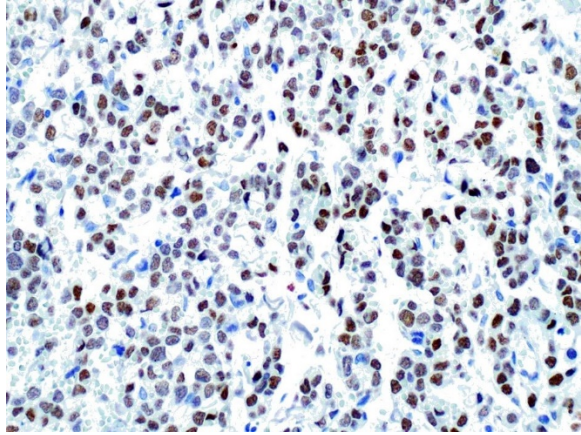
Lymphoma with plasmacytic differentiation include plasmacytoma, lymphoplasmacytic lymphoma and plasmablastic lymphoma. We compared the cellular morphology and immunohistochemical reaction between present case and those tumors with plasmacytic differentiation.

Typical plasmacytomas are composed of small to medium size round cells. The cells are characterized by moderate to slightly abundant cytoplasm with an eosinophilic or amphophilic appearance. Nuclei are round to

oval, small and irregular with single or multiple nucleoli and large, dense chromatin. Mitotic activity was varied but was usually low. The tumor cells are immunoreactive for CD79a, lambda and kappa light chains,



Haired skin, dog. Neoplastic cells are strongly immunopositive for CD-20, a B-cell marker. (Photo courtesy of Laboratory of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, Japan <http://www.setsunan.ac.jp/~p-byori/>)



Haired skin, dog. Neoplastic cells are positive for MUM-1, suggesting plasmacytic differentiation. (anti-MUM-1, 400X)

MUM-1^{7,16,18} and in a few cases are positive for CD20¹⁶.

Lymphoplasmacytic lymphoma is composed of small lymphocytes of a plasmacytoid type. The cells have characteristics of chronic lymphocyte lymphoma or lymphocytic lymphoma of intermediate type. The cells have small (slightly larger than erythrocyte) round nuclei; numerous small chromocenters, absent or small nucleoli and slight larger than scant cytoplasm. Rare immunoblasts are often present. The mitoses were very low. The tumor cells were immunoreactive for CD79a^{9,18}.

Plasmablastic lymphoma is a variant of diffuse large B-cell lymphoma, characterized by morphology and immunohistochemical criteria of plasmacytic differentiation. The cells are round or oval, of medium to large size with immunoblastic/centroblastic morphologic features or classic “plasmablastic” morphologic features, with scant to abundant cytoplasm, and a prominent central nucleolus or multiple smaller nuclei with chromatin clumped under the nuclear membrane. The mitoses were frequent. The cells do not express B cell makers such as CD20 and CD79a, but express of plasmacyte markers as MUM-1^{4,8}.

The present case has a cellular morphology similar to plasmablastic lymphoma, but

differed from it by immunohistochemical features. Thus, the present case was diagnosed as B cell lymphoma.

Canine cutaneous lymphoma is reported in about 3-8% of all canine lymphoma, and is histologically divided into epitheliotropic and non-epitheliotropic lymphoma^{5,7}. The majority of canine cutaneous non-epitheliotropic lymphomas are of T cell origin, B- cell origin is rare^{5,7}.

Animals with erythrophagocytic neoplasms in the spleen and liver often have non-regenerative anemia^{6,10-12,15,17}. In the present case, as the affected animal did not show obvious anemia at the time of a biopsy, it was suggested that our lymphoma may be localized in the skin.

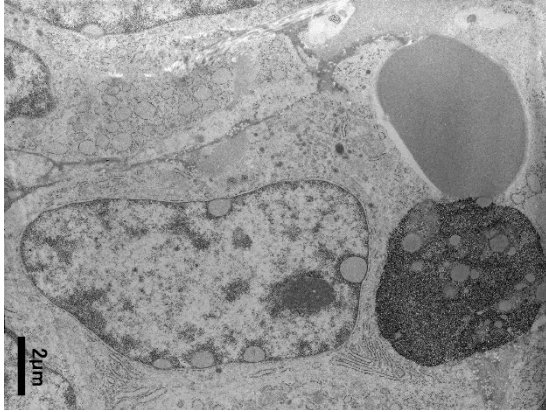
Contributing Institution:

Laboratory of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University,
45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, Japan

<http://www.setsunan.ac.jp/~p-byori/>

JPC Diagnosis: Haired skin: Plasmablastic lymphoma, large cell, high grade, plasmablastic, with marked erythrophagocytosis

JPC Comment: Conference participants agreed that the morphology of this unique neoplasm is that of a large cell (neoplastic cells exceed 2x that of an erythrocyte), high grade neoplasm. Identification of the cells on HE is difficult, especially given the degree of erythrocytosis exhibited by these cells. Conference participants identify significant plasmacytic differentiation among the neoplastic cells, although mature plasma cells were scattered throughout the neoplasm, as evidenced both on the HE stain and several immunostains run at the JPC (plasma cells commonly pick up immunostains in a non-



Haired skin, dog. Electron micrograph of a neoplastic B cell with two ingested erythrocytes within its cytoplasm. The one at left appears viable; significant degenerative changes including condensation occur within the one on the right. (Photo courtesy of Laboratory of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, Japan <http://www.setsunan.ac.jp/~p-byori/>)

specific fashion to frustrate pathologists.)

The CD-20 immunostain was repeated at the JPC and neoplastic cells was strongly positive; and a MUM-1 stain performed at UPenn was also positive. An IBA-1 stain demonstrated numerous infiltrating histiocytes. IBA-1 demonstrated some histiocytes within the neoplasm, but not a large component. Based on the immunohistochemical findings in this case, the moderator assessed this neoplasm as a likely B-cell neoplasm with early plasmacytic differentiation.

While we have seen cutaneous B-cell lymphoma in the skin numerous times, the erythrophagocytosis seen in this case is unique and remarkable. A Pubmed review of erythrophagocytic forms of lymphoma noted several cases of T-cell neoplasm exhibiting erythrophagocytosis in both humans and animals, this case appears unique for its B-cell erythrophagocytic activity. As B-cells do not have intracellular machinery to break down erythrocytes into hemosiderin and

lipofuscin like macrophages, this tumor is especially striking in the presence of apparently viable erythrocytes in neoplastic cells and a lack of hemosiderin pigment.

The moderator commented that while the presence of erythrocytes within neoplastic cells is unusual, it is prudent to believe in the conventional wisdom that neoplastic cells of any lineage may engulf erythrocytes if they desire to do so.

References:

- 1 Barger AM, Skowronski MC, MacNeill AL. Cytologic identification of erythrophagocytic neoplasms in dogs. *Vet Clin Pathol.* 2012;41:587-589.
- 2 Bertazzolo W, Dell'Orco M, Bonfanti U, et al. Canine angiosarcoma: cytologic, histologic, and immunohistochemical correlations. *Vet Clin Pathol.* 2005;34:28-34.
- 3 Carter JE, Tarigo JL, Vernau W, Cecere TE, Hovis RL, Suter SE: Erythrophagocytic low-grade extranodal T-cell lymphoma in a cat. *Vet Clin Pathol.* 2008;37:416-421.
- 4 Castillo JJ, Reagan JL. Plasmablastic lymphoma: a systematic review. *Scientific World Journal.* 2011; 11:687-696.
- 5 Day MJ. Immunophenotypic characterization of cutaneous lymphoid neoplasia in the dog and cat. *J Comp Pathol.* 1995;112:79-96.
- 6 Friedrichs KR, Young KM. Histiocytic sarcoma of macrophage origin in a cat: case report with a literature review of feline histiocytic malignancies and comparison with canine hemophagocytic histiocytic sarcoma. *Vet Clin Pathol.* 2008;37:121-128.
- 7 Gross TL IP, Walder EJ, Affolter VK. Lymphocytic tumors. In: *Skin diseases of the dog and cat.* 2nd ed. Oxford, UK: Blackwell; 2005:866-893.
- 8 Hsi ED, Lorsbach RB, Fend F, Dogan A: Plasmablastic lymphoma and related disorders. *Am J Clin Pathol.* 2011;136:183-

194.

9 Jaffe VE, Harris, N.L, Stein, H, Varidiman, J. W: Pathology & Genetic Tumor of Hematopoietic and Lymphoid Tissues. In: *World Health Organization Classification of tumours*. Lyon, France: 2001:120-134,171-174.

10 Kraje AC, Patton CS, Edwards DF. Malignant histiocytosis in 3 cats. *J Vet Intern Med*. 2001;15:252-256.

11 Ledieu D, Palazzi X, Marchal T, Fournel-Fleury C. Acute megakaryoblastic leukemia with erythrophagocytosis and thrombosis in a dog. *Vet Clin Pathol*. 2005;34:52-56.

12 Madewell BR, Gunn C, Gribble DH: Mast cell phagocytosis of red blood cells in a cat. *Vet Pathol*. 1983;20:638-640.

13 Marks SL, Moore PF, Taylor DW, Munn RJ. Nonsecretory multiple myeloma in a dog: immunohistologic and ultrastructural observations. *J Vet Intern Med*. 1995;9:50-54.

14 Matsuda K, Nomoto H, Kawamura Y, Someya Y, Koiwa M, Taniyama H. Hemophagocytic histiocytic sarcoma in a Japanese black cow. *Vet Pathol*. 2010;47:339-342.

15 Moore PF, Affolter VK, Vernau W. Canine hemophagocytic histiocytic sarcoma: a proliferative disorder of CD11d+ macrophages. *Vet Pathol*. 2006;43:632-645.

16 Ramos-Vara JA, Miller MA, Valli VE. Immunohistochemical detection of multiple myeloma 1/interferon regulatory factor 4 (MUM1/IRF-4) in canine plasmacytoma: comparison with CD79a and CD20. *Vet Pathol*. 2007;44:875-884.

17 Rossi S, Gelain ME, Comazzi S. Disseminated histiocytic sarcoma with peripheral blood involvement in a Bernese Mountain dog. *Vet Clin Pathol*. 2009;38:126-130.

18 Valli VE, Jacobs RM, Parodi, AL, Vernau W, Moore, P.F. *Hematopoietic Tumor of Domestic Animals*. vol. VIII. Wasington DC, USA: World Healt Organization

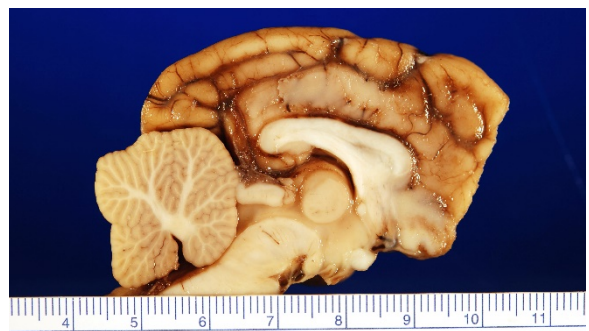
International Histological Classification of tumors of domestic animals; 2002:28-38.

19 Yearley JH, Stanton C, Olivry T, Dean GA. Phagocytic plasmacytoma in a dog. *Vet Clin Pathol*. 2007;36:293-296.

CASE III: 66619 (JPC 4066455).

Signalment: 12 year old, Male Neutered, Italian Greyhound, *Canis lupus familiaris*, Canine

History: One-week history of anxiety, nervousness, intermittent anorexia, and excessive vocalization. The vocalization was especially pronounced with regards to interactions involving the head or neck. The patient was referred to a neurologist for examination and further workup. On the morning of neurological consultation, an episode of ataxia, with a partial seizure occurred, followed by several hours of mental disorientation, the inability to sit up, and repetitive paddling activities impacting the entire left side of the body. When presented to the neurologist, the dog exhibited postural deficits on the right side.



Brain, dog: A focal 1.6x1.0 cm mass is present in the frontal lobe abutting the corpus callosum. (Photo courtesy of: Department of Molecular and Comparative Pathobiology, Johns Hopkins University, 733 N. Broadway, Suite 811, Baltimore, MD 21205, <http://www.hopkinsmedicine.org/mcp/index.html>)



Cerebral cortex, dog: A focal nodular well-demarcated neoplasm is present in the frontal lobe. (HE 4X)

Magnetic resonance imaging (MRI) showed a multifocal disease process impacting the falx cerebri rostral to the lateral ventricles and the ventrolateral right cerebrum adjacent to the meningeal surface. The ventrolateral lesion was approximately 1 cm in diameter with the falx cerebral lesions being smaller in size. Lesions coalesced at the cribriform plate.

Gross Pathology: Specimens submitted for histopathologic examination included brain (in entirety), nasal turbinate, lung, liver, spleen, and kidney. Gross examination of the brain revealed a focal extensive, 1.6 cm by 1 cm tan, firm, non-encapsulated, invasive mass in the gray matter of the frontal lobe abutting the corpus callosum, centered with an approximate midline – midline right orientation. Multifocally within the mass there are areas of malacia with a tan/red coloration (necrosis). The remainder of the submitted tissues are grossly unremarkable.

Laboratory results: NA

Microscopic Description:

Located in the grey matter of the frontal lobe is a non-encapsulated, poorly demarcated, multicentric, invasive mass of neoplastic round cells arranged in sheets and individual cells and supported on a pre-existing fibrovascular stroma. Neoplastic cells are round to oval with moderate eosinophilic finely vacuolated cytoplasm. Nuclei are

generally eccentrically located and round to reniform with coarsely stippled chromatin and up to three prominent nucleoli. There is marked anisocytosis and anisokaryosis, and mitoses number 1-3 per 40x field. Neoplastic cells are often multinucleate, with up to eight nuclei per cell. Neoplastic cells also frequently exhibit erythrophagocytosis. Throughout the mass, there are multifocally moderate numbers of infiltrating lymphocytes and plasma cells, and multifocal areas of necrosis. Multifocally, neoplastic cells invade and expand the meninges. No evidence of vascular or lymphatic invasion is appreciated.

Immunohistochemistry:

Kappa and Lambda Light Chain - Negative

CD3 – Negative

CD20 – Negative

GFAP – Negative

CD163 – Neoplastic cells exhibit variable cytoplasmic and membranous immunoreactivity

Iba1 – Neoplastic cells exhibit strong diffuse cytoplasmic immunoreactivity

Contributor's Morphologic Diagnoses:

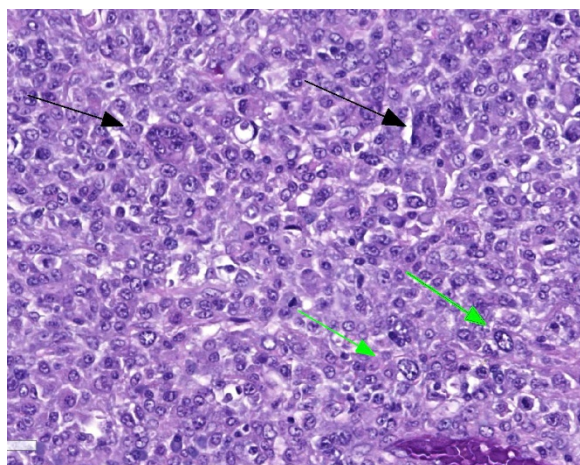
Brain (frontal lobe), primary malignant histiocytosis

Contributor's Comment:

Histologically, the mass in this dog's brain consisted of aggressive and highly invasive round cells with multicentric extension into the meninges. On immunohistochemistry, neoplastic cells were negative for T, B, and glial cell markers and positive for macrophage/monocyte markers (Iba-1, CD-163). Overall, immunohistochemistry results along with histopathological morphology are consistent with the diagnosis of malignant histiocytosis.

Histiocytic proliferative disorders can be subdivided into local and disseminated

forms. Localized forms are referred to as histiocytic sarcomas and are common primary tumors of the liver, spleen, tongue, and stomach wall in the canine patient.⁷ Malignant histiocytosis is the term most commonly used to describe disseminated neoplastic cells of histiocytic origin. The term “diffuse histiocytic sarcoma” has also been used to designate a localized histiocytic sarcoma that has become systemic. Additionally, benign aspects of histiocytic proliferative disorders include the cutaneous histiocytoma and cutaneous and systemic histiocytosis.



Cerebral cortex, dog: The neoplasm is composed of pleomorphic round cells. Multinucleated neoplastic cells (black arrows) and karyomegalic neoplastic cells, as well as mitotic figures are present in this field. (HE 400X)

In the canine patient, malignant histiocytosis most commonly occurs in middle age to older male dogs with increased incidence in the Bernese Mountain Dog, Rottweiler, Golden Retriever, and Labrador Retriever.⁵ Overall, the spleen and liver are the most commonly impacted tissues, but other organs including the lungs, lymph nodes, bone marrow, and central nervous system can also be impacted. Primary cases involving the brain are considered rare, with an overall poor to grave prognosis due to the invasive/aggressive nature of the neoplasia.⁷

As this case documents, definitive diagnosis of histiocytic tumors can be challenging with histopathology alone, and the use of immunohistochemistry can be advantageous. Overall, malignant histiocytic tumor histopathology is characterized by sheets of highly pleomorphic cells that exhibit severe atypia along with abundant mitotic figures. Noted throughout the neoplastic cells often are multinucleated giant cells, erythrophagocytosis, and scattered populations of lymphocytes and plasma cells. Malignant histiocytic tumors exhibit positive staining with CD1c, CD11c, CD45, lysozyme, CD18, and Iba1, and do not exhibit stain uptake for B cell, T cell, and glial cell markers.^{4,7} Iba1 is a 17-kDa EF hand protein that is specifically expressed in microglia and is not reactive with neurons or astrocytes. Iba1 has been recently recognized as a macrophage marker, expressed by all subpopulations of cells of the monocyte/macrophage lineage with efficacious used in the characterization of canine and feline histiocytic tumors.⁴

Differentials for this case included: a tumor of neuroglial origin, lymphoma, plasma cell tumor, and granulomatous disease.

Contributing Institution:

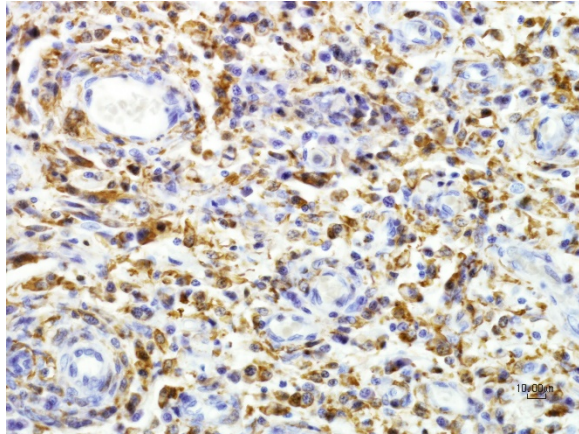
<http://www.hopkinsmedicine.org/mcp/index.html>

JPC Diagnosis: Cerebral cortex:
Histiocytic sarcoma

JPC Comment: The contributor has done an excellent job describing this rare entity in the dog. Disseminated histiocytic sarcomas (also referred to malignant histiocytosis) is not an uncommon neoplasm in the dog, but primary lesions in a single organ are.

A diagnosis of primary intracerebral histiocytic sarcoma not only requires immunophenotyping to establish the histiocytic lineage of the neoplastic cells, but

also to establish that no other similar lesions are present throughout the other tissues of the body. Recent studies in dogs have identified abnormalities in certain tumor suppressor genes (RB1, PTEN, and CDKN2A/B).³



Cerebral cortex, dog: Neoplastic cells are strongly immunopositive for IBA-1, a histiocytic marker. (anti-IBA-1 400X) (Photo courtesy of: Department of Molecular and Comparative Pathobiology, Johns Hopkins University, 733 N. Broadway, Suite 811, Baltimore, MD 21205 , <http://www.hopkinsmedicine.org/mcp/index.html>)

No gender or age predispositions have been identified for the development of primary intracerebral histiocytic sarcoma, and Pembroke Welsh Corgis have been identified as a predisposed breed.³

This particular case demonstrates the traditional appearance of a primary CNS histiocytic sarcoma – a single whitish subdural lesion within the cerebrum with a broad meningeal base. Secondary or metastatic lesions are usually multiple, and often arise within the meninges.² The subdural or meningeal location of these tumors may reflect the restriction of dendritic cells (the presumptive cell of origin for this neoplasm) to the meninges and choroid plexus.²

The moderator reviewed the maturation of cells of the macrophage/monocyte lineage as

well as common manifestations of histiocytic diseases in dogs and cats including canine cutaneous histiocytosis, reactive and systemic histiocytosis, feline progressive pulmonary histiocytosis, histiocytic sarcoma and the very rare dendritic cell leukemia.

References:

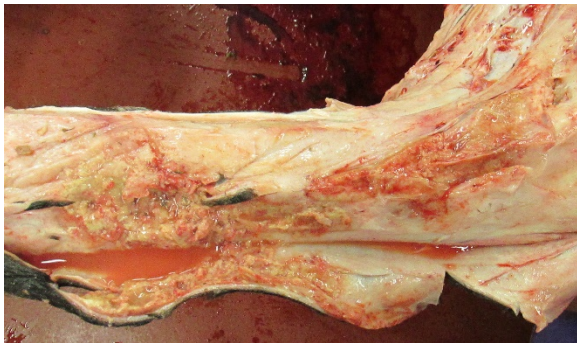
1. Chandra AM, Ginn PE. Primary malignant histiocytosis of the brain in a dog. *J Comp Pathol.* 1999;121:77–82.
2. Higgins RJ, Bollen AW, Dickinson PJ, Siso-Llonch S. Tumors of the nervous system. *In: Meuten DJ, ed. Tumors in Domestic Animals.* Ames, IA, Wiley Blackwell, 2017, pp. 877, 878.
3. Moore, PF. A review of histiocytic diseases of dogs and cats. *Vet Pathol* 2014;51:167-184.
4. Pierezan F, Mansell J, Ambrus A and Rodrigues Hoffmann A. Immunohistochemical Expression of Ionized Calcium Binding Adapter Molecule 1 in Cutaneous Histiocytic Proliferative, Neoplastic and Inflammatory Disorders of Dogs and Cats. *J. Comp. Pathol.* 2014, Vol. 151
5. Thio T, Hilbe M, Grest P and Pospischil A. Malignant Histiocytosis of the Brain in Three Dogs. *J. Comp. Path.* 2006, Vol. 134, 241–244
6. Uchida K, Morozumi M, Yamaguchi R, and Tateyama S. Diffuse Leptomeningeal Malignant Histiocytosis in the Brain and Spinal Cord of a Tibetan Terrier. *Vet Pathol* 38:219–222 (2001)
7. Zimmerman K, Almy F, Carter L, Higgins M, Rossmeisl J, Inzana K, Duncan R. Cerebrospinal fluid from a 10-year-old dog with a single seizure episode. *Veterinary*

CASE IV: UMC172 (JPC 4099791).

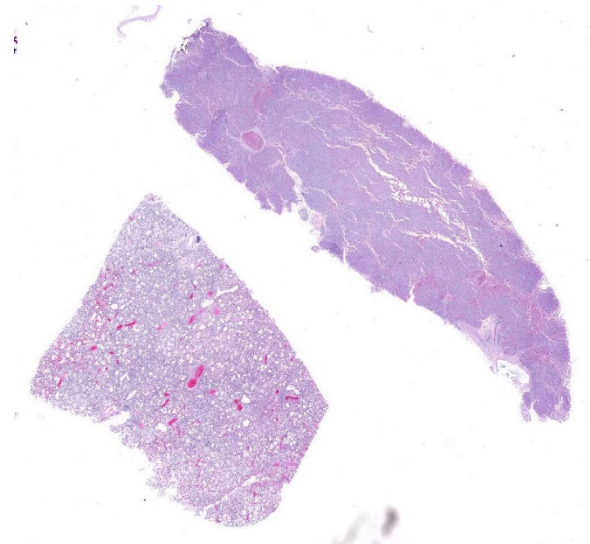
Signalment: 21 year old Thoroughbred gelding (*Equus caballus*)

History: This 21 year old Thoroughbred gelding was euthanized and submitted for necropsy due to a severe, chronic, non-healing wound on its right hind leg.

Gross Pathology: A 530kg body weight, aged adult, bay gelding, in moderate body condition (4/9 body condition score) is necropsied. The medial right hind limb is diffusely swollen from the inguinal region down to the level of the fetlock. A punctate fistula is noted on the medially, slightly below the fetlock joint. Considerable dense fibrous tissue is evident in the subcutis of the tibia and metatarsals. Roughly 100 ml watery translucent yellow orange fluid pools in the subcutis during dissection. Extending from the medial hock to the medial fetlock is soft, tan, friable tissue that oozes fluid. The process does not affect the bone, itself tendon sheaths or the joint fluids of the hock, fetlock or pastern. Other gross lesions include ulcers



Right rear leg, horse: The right front leg, when incised, demonstrates the extension of the chronic pyogranulomatous cellulitis. (Photo courtesy of: Veterinary Medical Diagnostic Lab and Department of Veterinary Pathobiology . <http://vmdl.missouri.edu/> <http://vpbio.missouri.edu/>)



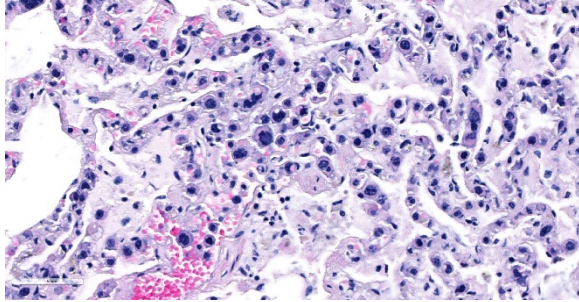
Liver, lung, horse: Sections of liver and lung are submitted for examination. (HE, 5X)

in the squamous portion of the stomach, and a large free-floating abdominal blood clot, thought to be a result of trauma sustained during euthanasia. The abnormalities in the organs submitted to the conference were unexpected.

Laboratory results: NA

Microscopic Description:

Large and small pulmonary vessels are congested, and there are contained numerous large nucleated cells located in alveolar capillaries. However, the cells are much less frequently present in larger arteries or veins. The cells have a defined outline and are polygonal in shape. They have large (even in relationship to cell size) polyhedral to reniform nuclei, with condensed chromatin. Occasional cells are multinucleate. Erythrophagia is common. Rare intravascular mitoses are found. In the liver, similar cells distend hepatic sinusoids, with atrophy of hepatic cords and degeneration. As in the lung, they have distinct cytoplasmic borders, large hyperchromatic nuclei and are erythrophagocytic. Additional similar



Lung, horse. Alveolar capillaries contain numerous large round cells with indented, often hyperchromatic nuclei. (HE, 400X)

cellular infiltrates were found in the spleen and in vessels but not in the parenchyma of the bone marrow.

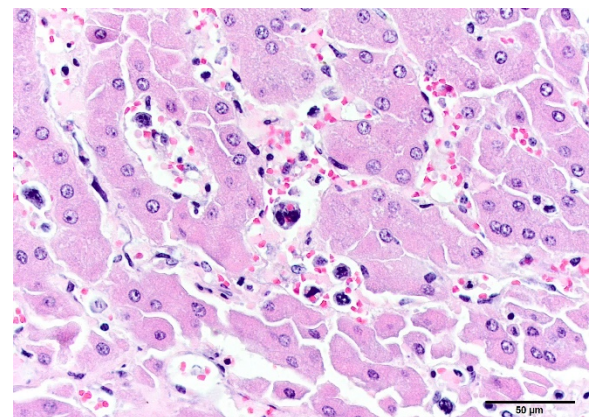
Immunohistochemistry identified the cells as CD3-positive. Positively staining cells have a linear arrangement in the marrow due to their intravascular location, which is not readily appreciated in the photo. CD20 staining identified a few small lymphocytes in the marrow itself. Numerous Iba-1 positive cells were present and appeared to be parenchymal, but co-localization of this marker with CD3 was not tested.

Contributor's Morphologic Diagnoses:
Lung and liver: Intravascular (angiotrophic) lymphoma, multiple organs

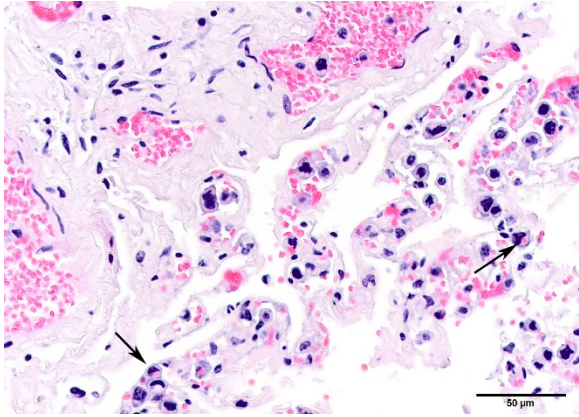
Contributor's Comment: Finding an intravascular lymphoma was entirely unexpected in this horse, which had been euthanized for intractable chronic cellulitis of one rear leg. Lymphoma is the most common malignant neoplasm of the hematopoietic system of horses, with the multicentric form being most common. B cell and T cell-rich B cell neoplasms are most common.¹ Angiotrophic or intravascular lymphoma, also call lymphoid granulomatosis, is one of the least common types of lymphoma in any species. Different types of lymphoma are

now considered specific diseases rather than a single entity.

Intravascular lymphoma is a rare disease and may be of B cell, T cell, or NK cell origin, in order of increasing rareness in people.^{2,4} Neoplastic cells are found only in small and medium-sized vessels, and it has been speculated that they cannot interact with the vascular wall to exit the circulation.² In people, especially in the western hemisphere, most cases are large B cell lymphomas. They are often difficult to diagnose hematologically. In Asian countries, the T cell phenotype is more common.⁴ T cell tumors have been associated with HIV infection.² Patients with intravascular lymphoma commonly present with fever, and fever due to this neoplasm might have been attributed to the leg infection in this horse. Hemophagia is very common, although in the past it had been attributed to red cell consumption macrophages in the tumor rather than tumor cells. No antemortem hematologic data was available for this patient. In our case, it is clear that the neoplastic cells are engulfing red cells. However, double immunolabeling was not done, and the possibility remains that this lymphoma could express both markers. Intravascular lymphoma cells typically reside



Liver, horse: Similar neoplastic cells are present within liver sinusoids and also exhibit erythrophagocytic activity. (Photo courtesy of: Veterinary Medical Diagnostic Lab and Department of Veterinary Pathobiology. <http://vmdl.missouri.edu/>)



Lung horse: Intravascular neoplastic lymphocytes occasionally exhibit erythrophagocytosis (arrows). (Photo courtesy of: Veterinary Medical Diagnostic Lab and Department of Veterinary Pathobiology. <http://vmdl.missouri.edu/>)

in small arteries veins and capillaries instead of large vessels;⁶ this characteristic was present in the horse. Intravascular or angiotropic lymphoma has been called by others a “homeless lymphoma” and apparently lacks the ability to exit the vasculature. It is thought that lack of β -integrin or ICAM homing receptor may explain the inability of these cells to exit the intravascular space.

This neoplasm has a short list of differential diagnoses, includes histiocytic sarcoma/leukemia, lymphoid leukemia and intravascular lymphoma. Intravascular lymphoma has been described in an Australian horse that presented with anemia due to hemophagic syndrome.⁴ Participants in this case were split between a diagnosis of leukemia or intravascular lymphoma, both of which are viable diagnoses in the absence of bloodwork or the ability to evaluate peripheral blood or bone marrow specimens. The moderator brought up an interesting clinical aspect of intravascular lymphomas in animals, as that peripheral blood smears of intravascular lymphoma are often devoid of neoplastic cells, while leukemias will show a

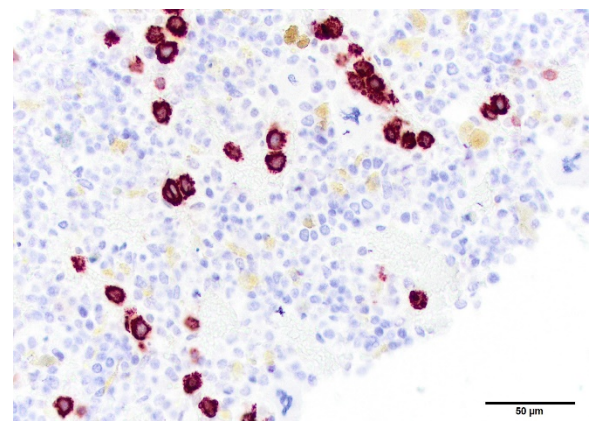
lymphocytosis as well as abnormal cells on a peripheral blood draw. Attendees also commented on the relative paucity of neoplastic cells in this particular case, while previous cases that they had seen (albeit all in dogs), contained numerous circulating neoplastic cells which often filled vessels in multiple organs. This may simply be a species difference.

The consensus of the participants is that the JPC diagnosis of “intravascular lymphoma with erythrophagocytosis” would be most appropriate in this case, as the poor preservation of the lung section as well as the pleomorphic nature of the cells precludes a more specific diagnosis in this case without the use of immunohistochemical markers.

Contributing Institution:

Veterinary Medical Diagnostic Lab and Department of Veterinary Pathobiology
<http://vmdl.missouri.edu/>,
<http://vpbio.missouri.edu/>

JPC Diagnosis: Lung, liver: Intravascular lymphoma, with erythrophagocytosis.



Bone marrow: CD3 staining of tumor cells. The linear arrangement is a result of intravascular localization. (Photo courtesy of: Veterinary Medical Diagnostic Lab and Department of Veterinary Pathobiology. <http://vmdl.missouri.edu/>)

JPC Comment: The contributor has supplied an excellent writeup of a unique case. Based solely on the morphology of the pleomorphic lymphocytes within the sinusoids and alveolar capillaries and their unfamiliarity with the syndrome in the horse, conference attendees preferred a diagnosis of intravascular lymphoma (IVL) until phenotyping information became available as the case was discussed.

Intravascular lymphoma is a rare entity in all species including humans. In humans, most cases of angiotropic are of B-cell origin. In the dog, in which the majority of this rare neoplasm has been reported, it is usually of T-cell origin. A previous case of cerebral intravascular lymphoma of presumptive T-cell origin was presented in WSC 2013-2014, Conference 6, case 2; dogs are also predisposed to develop this neoplasm within the nervous system.⁵

In the single previously reported case of IVL in a mare, antemortem diagnosis was not made, a common finding in IVL likely resulting of the variable involvement of somewhat random organs often seen in this condition. In that report, a mare with a viable 8-month fetus rapidly developed extravascular hemolysis and regenerative anemia as a result of erythrophagocytosis by an activated reticuloendothelial system (hemophagocytic syndrome), rather than by neoplastic cells as seen in this case.

A possible reason for the intravascular nature of the neoplastic cells comes from human cases, in which neoplastic B cells have demonstrated deficiencies in β_1 - (CD29) or β_2 -integrin (CD18) deficiencies. β -integrins are important in the leukocyte adhesion cascade, enabling leukocytes to exit blood vessels and migrate through tissue in order to participate in inflammation. The lack of these molecules may render neoplastic

lymphocytes unable to exit vessels, resulting in high numbers within the vasculature.⁵

References:

1. Valli VEO, Kiupel M, Bienzle D. Hematopoietic System. In Maxie G, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals Vol 3*, 6th ed. Elsevier, Inc. St. Louis; 2016:237-238.
2. Ponzoni M, Ferreri AJM. Intravascular lymphoma: a neoplasm of "homeless lymphocytes. *Hematol Oncol.* 2006;**24**:105-112.
3. Raidal SL, Clatk, P, Raidal SP. Angiotropic T-cell lymphoma as a cause of regenerative anemia in a horse. *J Vet Intern Med.* 2006;**20**:1009-1013.
4. Sukpanichnant S, Visuthisakchai S. Intravascular lymphomatosis: a study of 20 cases in Thailand and review of the literature. *Clinical lymphoma & Myeloma.* 2006; **6**(4):319-328.
5. WSC 2013-2014, Conference 6, Case 2; http://www.askjpc.org/wsc/wsc_showcase4.php?id=djZ2eEQwNDZoc1NZZUNDTXB5R3IUZz09
6. Yao X, Sadd A, Chitambar CR. Intravascular B-cell lymphoma, and exclusively small vessel disease? Case report and review of the literature. *Leuk Res.* 2010;**34**:275-77.