UNCLASSIFIED MALIGNANT ROUND CELL TUMOR IN A JUVENILE DOG: A CANINE COUNTERPART OF SMALL ROUND CELL TUMOR OF CHILDHOOD?

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Conclusions

- A cell of origin of this dog's tumor could not be determined, and there was no distinct correlation with known human round cell neoplasms.
- This case represents a rare event and supports the implementation of wider immunohistochemical screening and molecular diagnostic methods, though methods used for human cases may have a lower efficacy rate or different outcomes.

Patient's signalment

Border Collie dog, intact male, 2-year-old

Clinical course

28 days from first presentation to death.

January 24, 2014 — Presented to regional veterinary clinic for right pelvic limb lameness. Right tibial osseous neoplasm was suspected by radiograph.

January 25, 2014 — Presented to Japan Small Animal Cancer Center for further examination and treatment. Computed tomography revealed a destructive mass in the right proximal tibia, right popliteal and subiliac lymphadenopathy, and multiple renal cysts. Cytology of the tibial mass identified differential diagnoses of osteosarcoma and poorly-differentiated carcinoma.

January 30, 2014 — Performed right pelvic limb amputation. Histopathology of the tibial mass was diagnosed as malignant neoplasm of undetermined origin with metastasis to the right popliteal and inguinal lymph nodes. The tumor cell did not react with CK AE1/AE3, vimentin, CD3, CD20, MHC2, chromogranin A, c-KIT by immunohistochemistry (IHC).

February 10, 2014 — Discharged from the referral clinic.

February 12, 2014 — Decreased appetite.

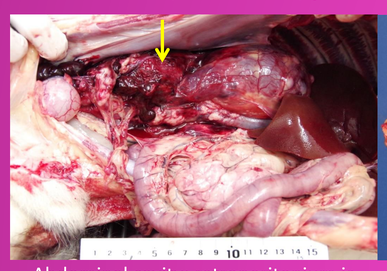
February 19, 2014 — Lost appetite, renal failure.

February 20, 2014 — Oliguria, death.

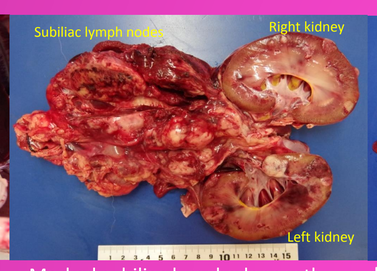
Performed **autopsy** 2.5 hours after death. Nasal cavity, spinal cord, joint cavities were not examined.

Summary of autopsy findings

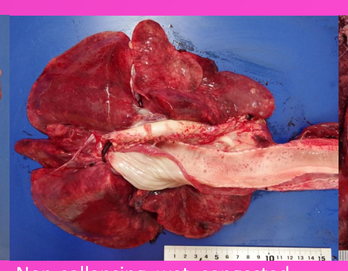
- **Subiliac and hilar lymph nodes, both kidneys, urinary bladder, lung, mesentery, cerebrum**: neoplasm
- **Kidneys and ureters**: hydronephrosis and hydroureter
- **Lung**: diffuse alveolar damage, suspected
- **Heart**: epicardial and endocardial hemorrhage
- **Thoracic, abdominal, pericardial cavities**: effusion



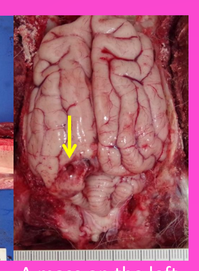
Abdominal cavity: retroperitonium is expanded by severely enlarged subiliac lymph nodes (arrow).



Marked subiliac lymphadenopathy, multiple renal masses, and hydronephrosis.



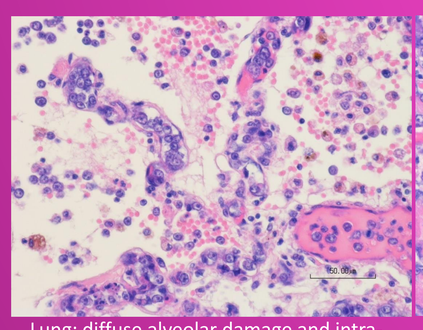
Non-collapsing, wet, congested, heavy lung. The trachea is filled with foamy fluid.



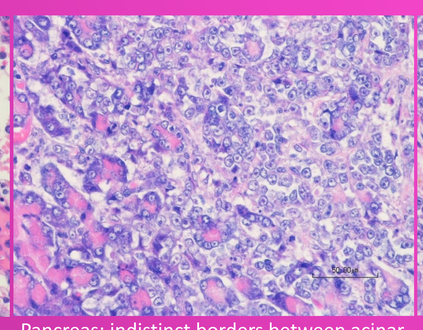
A mass on the left parietal lobe (arrow).

Summary of histopathological findings

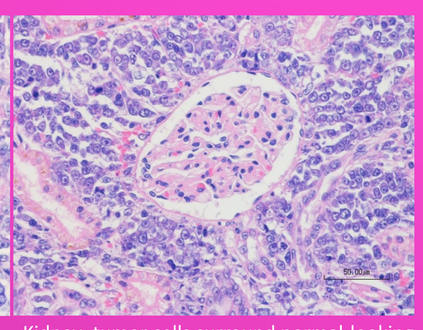
- **Lymph nodes (subiliac, mesenteric, splenic, hilar), kidneys, urinary bladder, lung, cerebrum, pituitary gland, adrenal gland, pancreas, surgically-excised right tibial mass**: disseminated round cell tumor of undetermined origin with vascular/lymphatic invasion
- **Lung**: diffuse alveolar damage
- **Kidney**: severe congestion
- **Heart**: multifocal hemorrhage and necrosis
- **Liver**: centrilobular congestion, multifocal hemorrhage, hepatocellular degeneration
- **Spleen**: microhemorrhage
- **Diaphragm**: focal hemorrhage



Lung: diffuse alveolar damage and intra-vascular tumor cells.



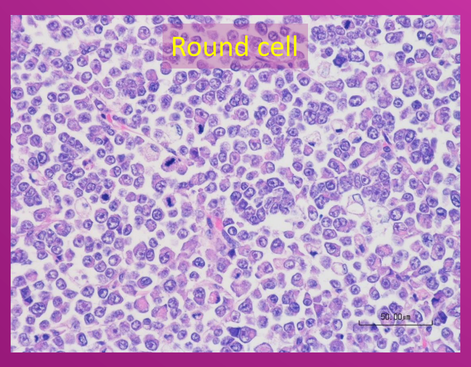
Pancreas: indistinct borders between acinar cells and tumor cells suggesting pancreatic origin of the tumor.

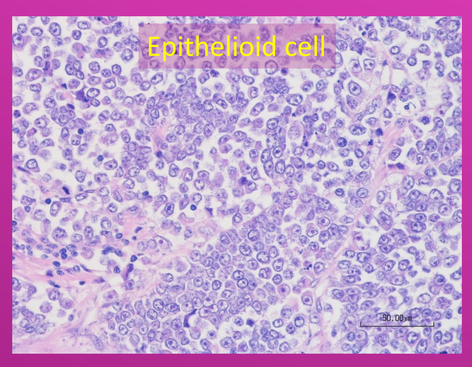


Kidney: tumor cells surround normal-looking glomerulus.

Histologic features of the tumor cell

- Atypical polyhedral (epithelioid) or round cells proliferate in sheets and nests with delicate to small amount of stroma.
- Distinct cell boundaries, moderate amount of weakly basophilic fine granular cytoplasm, round to ovoid single vesicular nuclei with slight anisokaryosis (3 to 5 red blood cells nuclear diameter), prominent single nucleoli.
- Mitotic figures 40/10 HPF.
- Frequent lymphatic/vascular invasion and geographic necrosis.

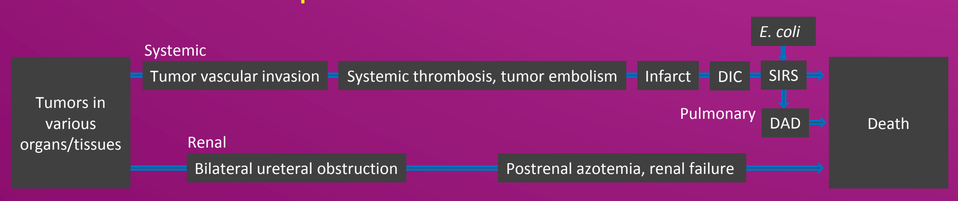




Results of bacterial culture

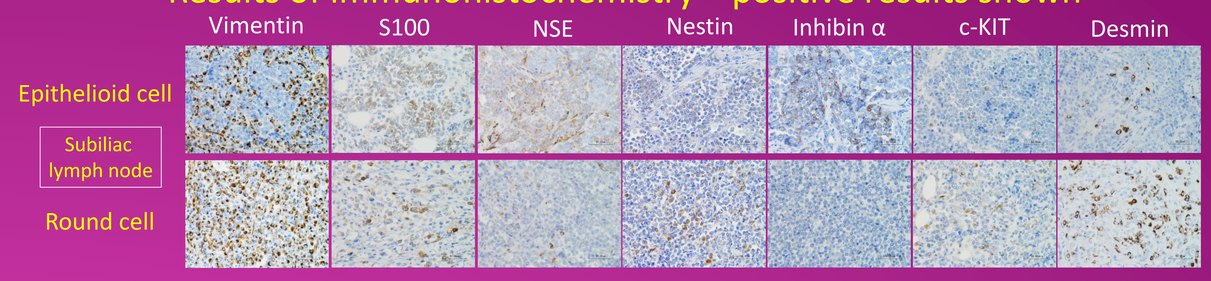
Escherichia coli grew from pulmonary swab specimen.

Proposed mechanism of death



Abbreviations; DAD: diffuse alveolar damage, DIC: disseminated intravascular coagulation, SIRS: systemic inflammatory response syndrome

Results of immunohistochemistry – positive results shown



Summary of immunohistochemistry and histochemistry results

Antibody/stain	Target	Subiliac LN		Pancreas
		Epithelioid cell	Round cell	(mostly round cell)
Vimentin	Various mesenchymal cells	−/+	+/−	+/−
S100	Various nerve cells, chondrocytes, melanocytes	+/−	−/+	ND
NSE	Various nerve cells	+/−	R	+/−
Nestin	Progenitor glial cells in the CNS, Schwann cells in the PNS, early myoblast/myotubes	−/+		R
Inhibin alpha	Sex-cord stromal cells, adrenocortical carcinomas	−/+	N	R
CD117 (c-KIT)	Mast cells, Cajal cells, etc.	R	−/+	ND
Desmin	Various muscle cells	R	−/+	ND
Cytokeratin AE1/AE3	Various epithelial cells	N		
CD3	T lymphocytes	N		ND
CD20	B lymphocytes	N		ND
Iba-1	Microglia, macrophages	N		ND
CD163	Macrophages	N		
MUM1	Plasma cells	N		ND
E-cadherin	Langerhans cells	N		ND
EMA	Various epithelial cells	N		ND
WT-1	Wilms tumors	N		ND
GFAP	Astrocytes	N		ND
myoD1	Rhabdomyosarcoma	N		ND
HHF35 (muscle specific actin)	Various muscle cells	N		ND
PGP9.5	Various neuroendocrine cells, neurons	N		ND
MelanA	Melanocytes, adrenal cortex, ovarian follicles, testicular interstitial cells	N		
Synaptophysin	Various neuroendocrine cells	N		
Chromogranin A	Various neuroendocrine cells	N		
Trypsin	Pancreatic acinar cells	No cross-reactivity to canine tissue, likely		
MIC2 (CD99)	Ewing sarcoma/primitive neuroectodermal tumor, lymphoblastic lymphoma	No cross-reactivity to canine tissue, likely		
Chymotrypsin	Pancreatic acinar cells	No cross-reactivity to canine tissue, likely		
α-Amylase	Pancreatic acinar cells	N		
Amylase	Pancreatic acinar cells	N		
Lipase	Pancreatic acinar cells	No cross-reactivity to canine tissue, likely		
Insulin	Islet β cells	ND		N
Glucagon	Islet α cells	ND		N
Toluidine blue stain	Mast cell granules	No heterochromatic granules		ND
Reticulin silver stain	Demonstrate encasement of epithelial tumor cell clusters by fibrous tissue	No encasement of tumor cell		ND

+, almost always diffuse strong positivity; +/−, variable staining, mostly positive; −/+, variable staining, mostly negative; R, rare cells positive; N, almost always negative; ND, not done.

Discussion

Apparent atypical characteristics and disseminated proliferation of the tumor cells precluded the possibility of benign neoplasm. The eliminated differential diagnoses by cytomorphology, pattern of proliferation, features of stroma, results of IHC and histochemistry are as the following:

- Discrete round cell tumor: lymphoma/leukemia, plasmacytoma, histiocytic sarcoma, mast cell tumor
- Tumors originating from retroperitoneal structures: adrenocortical carcinoma, malignant pheochromocytoma, neuroblastoma
- Pancreatic tumor: acinar cell carcinoma, malignant islet cell tumors, pancreatoblastoma
- Others: malignant melanoma, osteosarcoma, seminoma, poorly-differentiated carcinoma

The clinicopathological features of the patient's tumor suggest malignant small round cell tumor (MSRCT) of childhood. MSRCT typically includes rhabdomyosarcoma, lymphoma, Wilms tumor (nephroblastoma), leukemia, Ewing sarcoma/primitive neuroectodermal tumor (ES/PNET), desmoplastic small round cell tumor, malignant rhabdoid tumor (MRT), and osteosarcoma.[1] Cytomorphological similarities among these human tumors necessitate IHC by a panel of antibodies and genetic analysis on top of routine histopathology for a definitive diagnosis. The table below denotes main criteria for diagnosing human MSRCTs and discrepant point(s) of the patient's tumor to those criteria.[1]

Malignant small round cell tumor of childhood

	Main criteria for diagnosis	Discrepant point(s) of the patient's tumor
Rhabdomyosarcoma	● Multiple histologic subtypes exist (embryonal, botryoid, alveolar, pleomorphic, spindle cell/sclerosing) ● Positive IHC for myogenic markers (myogenin, MyoD1, desmin)	● Histologic features ● IHC results
Wilms tumor (nephroblastoma)	● Blastemal, epithelial, and stromal components exist simultaneously or in combination or solely ● Positive IHC for WT1 and Pax-2 of blastemal cells	● Histologic features
Neuroblastoma	● Positive IHC for NSE, CD56, CD57, PGP9.5, synaptophysin, chromogranin, etc. ● Lacks expression of vimentin, desmin, myoD1, keratins, CD99, etc.	● IHC results
ES/PNET	● Arises in bone, soft tissue, or primary site cannot be determined ● Positive IHC for vimentin, CD99, FLI1 ● Cytogenetic and molecular genetic tests are available	● IHC results, especially negative results for CD99
Desmoplastic small round cell tumor	● Positive IHC for vimentin, cytokeratin, EMA, desmin, WT1 ● Negative for myogenic markers ● Cytogenetic and molecular genetic tests to detect EWSR1-WT1 gene fusion are available	● IHC results
MRT	● Very aggressive and dissemination is common ● Diffuse positivity for vimentin, focal positivity for at least one epithelial marker, varied expression of mesenchymal and neuroectodermal markers ("polyphenotypic")	● IHC results, especially negative results for epithelial markers
Osteosarcoma	● Presence of osteoid is the key feature	● Histologic features

(discussion continues)

Among MSRCTs, ES/PNET and MRT could still be in the list of differential diagnoses for this dog's tumor since there are some overlaps in IHC profiles. Tumor's disseminated proliferation and uncertainty on cell/tissue of origin also contribute to this idea.

Though an incident of Ewing sarcoma of a dog was reported in 1969 (the article is in German), the credibility of this diagnosis has been questioned due to the fact that it was well before the era of the extensive use of IHCs and molecular diagnostics.[3] While CD99 positivity is a prerequisite for diagnosis of ES/PNET, we, however, saw no reaction of human CD99 marker to canine normal lymphocyte, which seems indicative of an inherent problem in using this marker to investigate possible canine cases of ES/PNET.

A case of MRT was reported in 1997 in a 1.5-year-old female Border Collie dog.[4] The tumor was found in the right piriform lobe of the brain, which was submitted independently for rabies test. Tumor cells of this dog reacted diffusely to vimentin and scatteredly to NSE and GFAP. Examination of other organs/tissue was not performed thus distribution of the tumor cell was unknown.

Of note, reactivity to nestin, which is a marker for neuroepithelial stem cells, can be used to differentiate MRT (positive) from ES/PNET (negative) in human cases.[2] If this works in similar way for canine cases, the tumor of this dog can be classified as MRT because of its reactivity to nestin. However, making a definitive diagnosis through MRT still needs another positive IHC reaction to at least one of multiple epithelial markers such as CK, EMA, CAM5.2, and so on. Since CAM5.2 was not available in our investigation, further trial for additional IHCs using other epithelial markers is warranted.

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4. Steele KE, Schulman FY, Mena H, et al. Rhabdoid tumor in the brain of a dog. *Vet Pathol*. 1997; 34:359-363.