ESVCP/ECVCP Mystery Case 2019

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SPECIMEN: Nodule on left tarsus, aspirate, Modified Wright stain

SIGNALMENT: Canine, 7-year-old, male, castrated Labrador Retriever, 32.4 kg

HISTORY AND CLINICAL FINDINGS: The patient was presented to Purdue University Veterinary Teaching Hospital (PUVTH) for evaluation of a seven-month history of an intermittent non weight bearing lameness on his left hind limb. On physical examination, a slightly enlarged left popliteal lymph node was noted and a painful, small nodule was found on the medial aspect of his tarsus. Radiographs revealed a small, rounded, opaque, soft tissue nodule superimposed with the soft tissues plantar to the calcaneous without osseous involvement. Ultrasound was performed and an irregularly marginated anechoic to hypoechoic nodule with hyperechoic foci was described. A fine needle aspirate was taken and submitted for cytological evaluation. Additional findings included history of controlled diabetes mellitus, mature cataract, and anterior uveitis on both eyes. His CBC was unremarkable and chemistry panel (Vitros 5,1 FS Chemistry System, Ortho-Clinical Diagnostics, Raritan, NJ, USA) had a few changes presented below.

TEST		UNITS	REFERENCE INTERVAL
Total Protein	7.1	g/dL	4.8-6.9
Albumin	4.3	g/dL	2.3-3.9
ALT	98	IU/L	3-69
ALP	641	IU/L	20-157

Serum sample displaying moderate hemolysis and marked lipemia.

CYTOLOGICAL IMAGES:

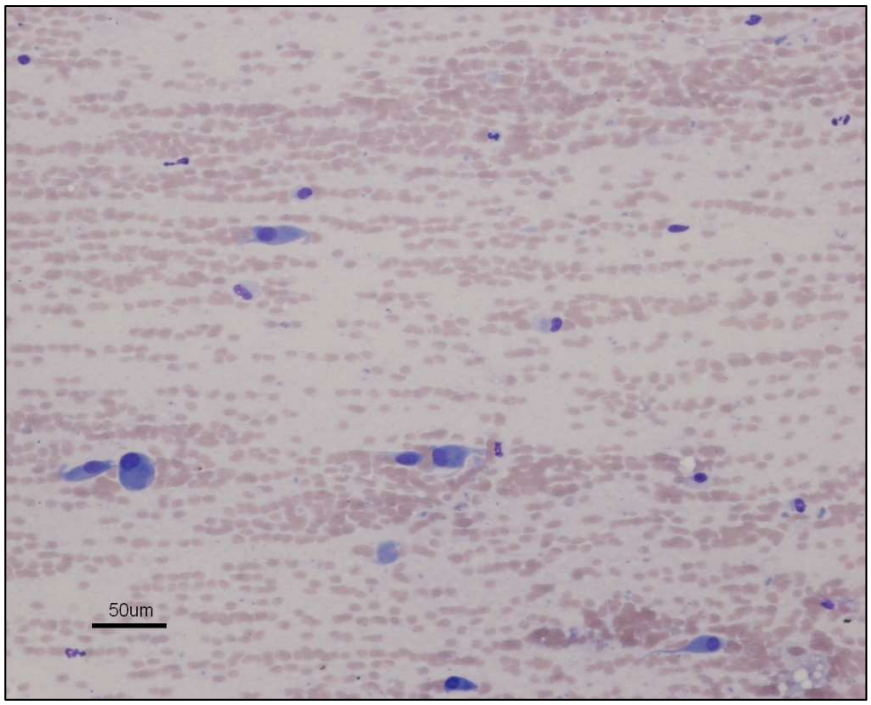

Figure 1: Overall cellularity of the nodule aspirate smear. Moderate numbers of round to spindle cells in a background with moderate hemodilution and remarkable windrowing are observed. Modified Wright stain (20x objective).

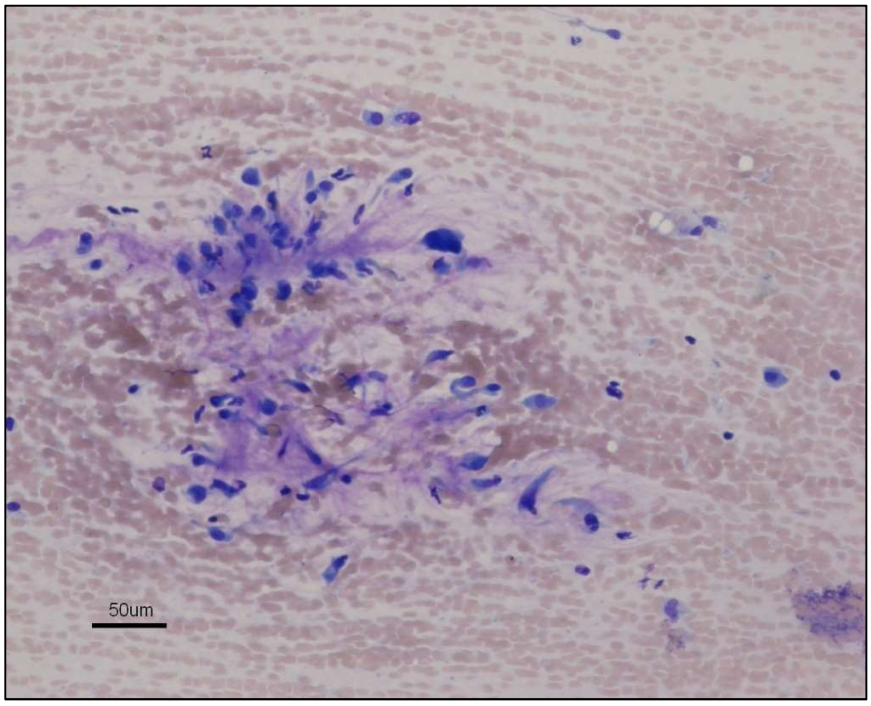

Figure 2: Occasional cells are embedded in a pink amorphous matrix. Nodule aspirate smear. Modified Wright stain (20x objective).

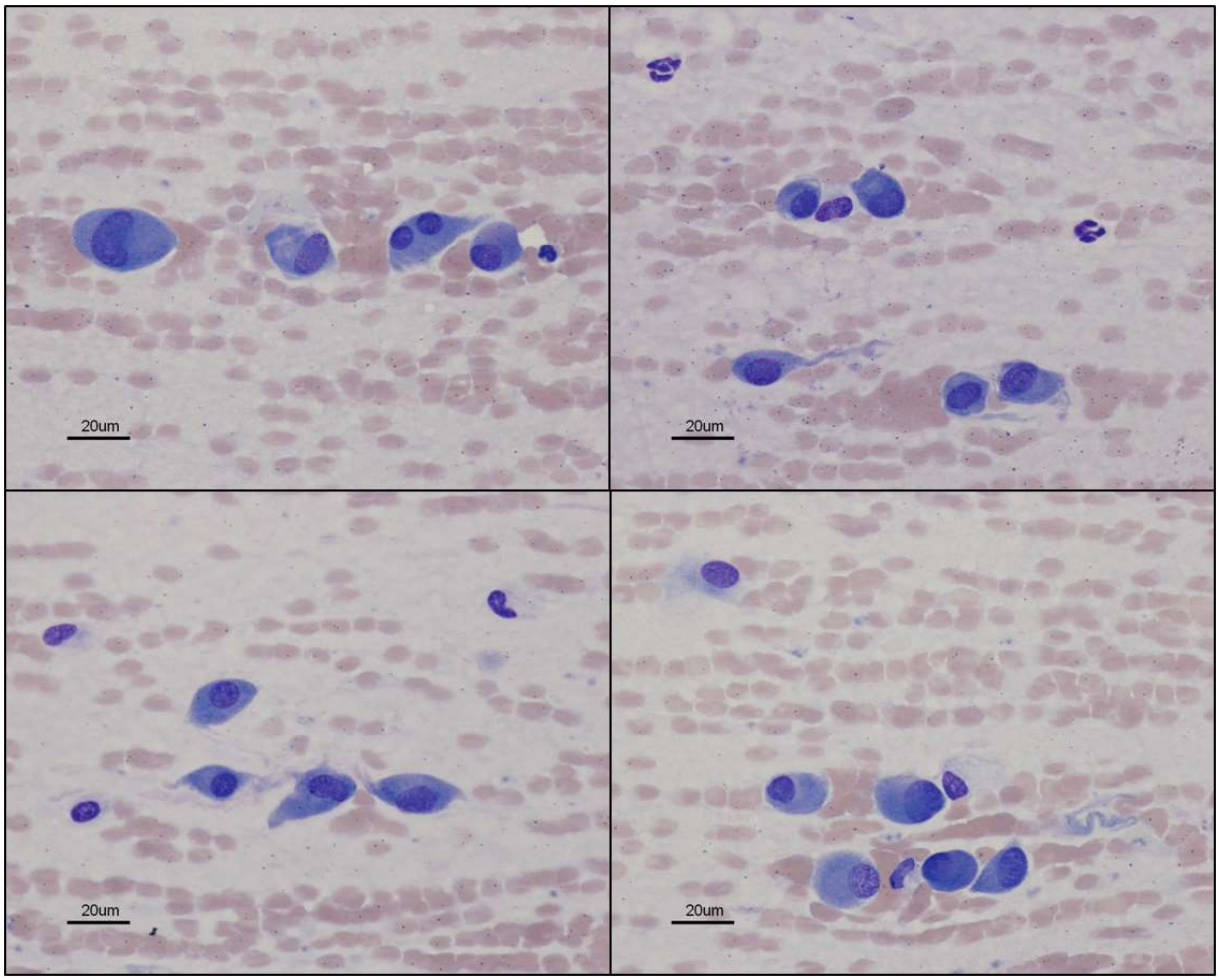

Figure 3: The cells present in the aspirate display anisocytosis, anisokaryosis, variable nuclear shape, and binucleation. Nodule aspirate smear. Modified Wright stain (60x objective).

QUESTIONS:

1. Based in the overall cellularity, background, and morphology of the nucleated cells present in the nodule aspirate, what is your primary differential diagnosis?
    a. Histiocytic sarcoma
    b. Myxoma/myxosarcoma
    c. Osteosarcoma
    d. Plasma cell tumor
    e. Soft tissue sarcoma

CYTOLOGICAL DESCRIPTION: The specimen is moderately cellular, consisting of a neoplastic population of cells in a background of moderate blood contamination. The cells are round to spindloid, with moderate to marked anisocytosis, and moderate N:C ratios. They are present mostly individualized, with rare cells embedded in a pink amorphous proteinaceous matrix. The cytoplasm is blue and occasionally contains a perinuclear clearing zone. The nuclei are round to oval, occasionally reniform, with finely stippled to reticular chromatin patterns. The nuclei display moderate anisokaryosis. A few binucleate cells are noted. One round, large, prominent nucleolus is occasionally seen. Low numbers of activated macrophages and mast

cells are observed. Marked windrowing is observed. No infectious agents are identified (**Figures 1-3**).

CYTOLOGICAL INTERPRETATION: Most consistent with malignant neoplasia, minimal macrophagic inflammation, and minimal mast cell infiltration.

COMMENTS: Differential diagnoses include soft tissue sarcoma, histiocytic sarcoma, plasma cell tumor, myxosarcoma, and synovial sarcoma. The presence of windrowing indicates increased viscosity and it can be related to accidental puncture of the underlining joint, communication of the nodule with the joint, or production of mucinous material by the neoplastic cells. Histopathologic evaluation is highly recommended.

ADDITIONAL FINDINGS: Approximately 30 days after his initial presentation, the dog returned for Magnetic Resonance Imaging (MRI), nodule punch biopsy with histopathological examination, and left popliteal lymph node fine needle aspiration. The MRI revealed a 2 x 1.5 x 0.8 cm soft tissue nodule and no joint involvement. The left popliteal lymph node consisted of peripheral blood and was non-diagnostic. On histopathology, a spindle cell tumor located under unremarkable haired skin was described. As in the cytology, the neoplastic cells are round to fusiform, with basophilic to eosinophilic cytoplasm, variable-sized oval nuclei with variable chromatin pattern and three mitotic figures per 10 high power fields. The mass has a fibrous to myxomatous stroma. The morphologic diagnosis was soft tissue sarcoma. Immunohistochemistry was also performed in the mass. Antibodies for vimentin, actin muscle, smooth muscle actin, S100, CD18, and Alcian blue stain were requested (**Figure 4**). The neoplastic cells were strongly positive for vimentin, moderately positive for actin muscle, focally positive for smooth muscle, and negative for the remaining markers. The stroma stained positive for Alcian blue, confirming the mucin, which was observed in both cytological and histological preparations.

DIAGNOSIS: Myxoid rhaddomyosarcoma

CLINICAL OUTCOME/FOLLOW-UP: The owners opted for palliative radiation therapy instead of excisional removal of the mass followed by chemotherapy or limb amputation. The goal is to stabilize and reduce the size of the tumor, to relieve pain, and to improve the patient's quality of life. Until this summary was written, the patient was stable and had his first appointment for radiation therapy scheduled.

ANSWERS TO QUESTIONS: 1) a

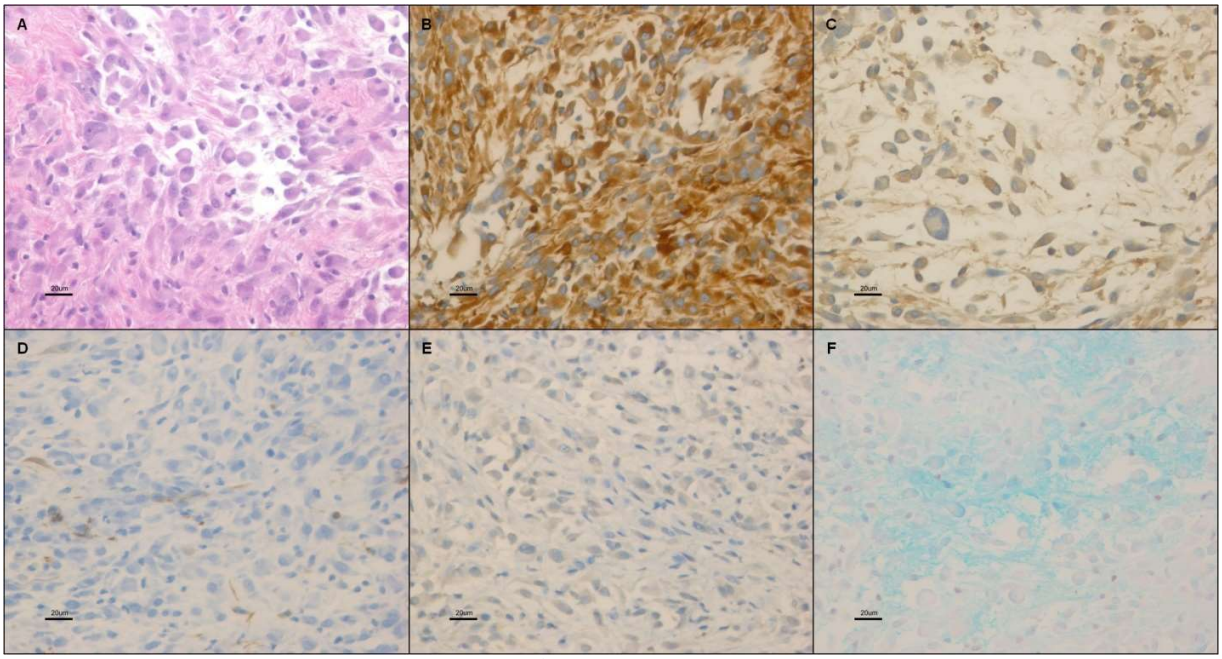

Figure 4: Punch biopsy sample submitted for histopathological evaluation. Hematoxylin-eosin stain (A), vimentin (B), actin muscle (C), smooth muscle actin (D), S100 (E), and Alcian blue (F) are present in the picture (60x objective).

DISCUSSION:

Canine soft tissue sarcomas (STSs) are neoplasms of mesenchymal origin derived from soft connective tissues and can occur in any part of the body. They are considered as a group of tumors given their similar microscopic and clinical features. In Veterinary Medicine, STSs have become a diagnostic of exclusion, but the use of this term is not consistent between pathologists. Approximately, 8-15% of all cutaneous and subcutaneous tumors in dogs are classified as STSs (Dennis et al. 2011).

The cytomorphology of the neoplastic cells in this dog defined the classification as malignant. In this case, the presence of matrix and high viscosity could lead towards the diagnosis of myxosarcoma; however, there are only focal areas positive for Alcian blue in this mass (Riegel et al. 2008). A second possibility would be a synovial sarcoma. In humans, synovial sarcomas usually contain focal areas of pan-cytokeratin positive cells. A few cases of biphasic synovial sarcomas have been described in dogs, but given the unknown cell origin of this kind of neoplasia, its controversial the use of this nomenclature (Craig et al. 2002; Monti et al. 2018).

The use of immunohistochemistry (IHC) has helped with the classification of STSs when cellular pleomorphism does not allow a phenotypic distinction. After the morphologic diagnosis of soft tissue sarcoma, the IHC antibodies were chosen based in possible differentials. Vimentin was chosen to confirm mesenchymal origin. Actin muscle was chosen for confirmation of rhabdomyosarcom and smooth muscle actin for leiomyosarcoma and pleomorphic sarcoma. S100 was performed to rule out peripheral nerve sheath tumor and perivascular wall tumor, and CD18 for histiocytic sarcoma (Chijiwa et al., 2004; Dennis et al., 2011; Williamson & Middleton, 1998). Lastly, Alcian blue was chosen to confirm the myxoid nature of the matrix. The presence of myxoid matrix resembles the description of an unusual case of a 14-year-old dog diagnosed with embryonal rhabdomyosarcoma (Cooper & Valentine, 2017). Other soft tissue mesenchymal tumors were ruled out; liposarcoma, based in lack of lipocytes and lypoblasts; hemangiosarcoma, based in lack of vascular structures within the mass; and fibrosarcoma,

based on the lack of interwoven bundles and pronounced collagenous stroma. However, these tumors do not necessarily express all their histological hallmarks (Hendrick, 2017).

Based in the positivity for actin muscle, the final diagnosis of rhabdomyosarcoma appeared to be the most appropriate in this case, even though no strap cells were identified and a complete list of immunohistochemistry markers was not performed. No pan-cytokeratin, MHC II, von Willebrand factor, and desmin, were run in this case, which may represent potential limitations for further classification of this tumor as synovial sarcoma, histiocytic sarcoma, hemangiosarcoma, and to provide stronger evidence of rhabdomyosarcoma, respectively (Hendrick, 2017).

In the chemistry panel, the mild hyperproteinemia and hyperalbuminemia are most likely related to hemoconcentration. The mild elevation in ALT can be an indicative of mild hepatocellular injury, although a muscle injury caused by the lameness cannot be completely ruled out without a CK measurement. The mildly increased ALP without concomitant increase in total bilirubin and GGT likely represent corticosteroid stress and less likely early cholestatic process related to the patient's diagnosis of diabetes.

According to the current classification for STSs in dogs, the present sarcoma is of low grade. Complete mass removal with wide or radical excision is associated with better clinical outcome (Dennis et al. 2011). However, the location of the mass in this case precludes removal, and amputation was denied by the owner. Radiation therapy alone may result in good outcome given the small size of the nodule.

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