DIAGNOSTIC EXERCISE

From The Davis-Thompson Foundation*

Case #:**253**; Month: **January**; Year: **2025**

Answer Sheet

Title: Lymphoproliferative disorder with plasmacytoid differentiation in a dingo (*Canis familiaris*)

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Clinical history: A free-ranging 10-year-old male neutered tan to brown, off-white with black spots dingo (*Canis familiaris*) living in a regional wildlife park was found dead in his den. Two months prior to his death, multiple body lumps were palpated: a 2.0x2.0 cm soft nodule on the left upper abdomen, 1.0x1.0 cm firm nodule on the central abdomen and a 2.0x2.0 firm nodule on the lumbar spine. Lumps were clinically suspected of lipoma, but FN samples were non diagnostic. Additionally, he had mild alopecia in the distal pinnae, which was negative for any infectious agents. The animal was monitored and didn't show signs of illness prior to death.

Post-mortem findings:

This dingo presented a 6/9 body score condition and mild dehydration. On a mid-diaphyseal tangential section of multiple bones, the bone marrow displayed increased reddened tissue in proportion to the adipose tissue. The cranial aspect of the left lateral hepatic lobe was expanded by a raised, firm, off-white, sessile, circumferential mass with centrally depressed, highly friable, dark red focus. Expanding the central aspect of the same hepatic lobe is a circumferential, slightly raised mass peripherally light red with an irregularly delineated dark red core. Approximately 70% of the right ventricular wall was expanded and replaced by multiple, firm, off-white nodular, frequently coalescing masses ranging from 0.5 to 4 cm diameter. The right ventricular to left ventricular ratio is 1:2 (ventricular volume overload and dilatation). Other gross findings included the presence of luminal dark red frothy liquid in the trachea (oedema), pulmonary rib impressions and oozing of dark red fluid on the pulmonary cut surface (oedema and congestion), a 1-2 cm lipoma located on the central abdominal subcutis.

Gross picture:

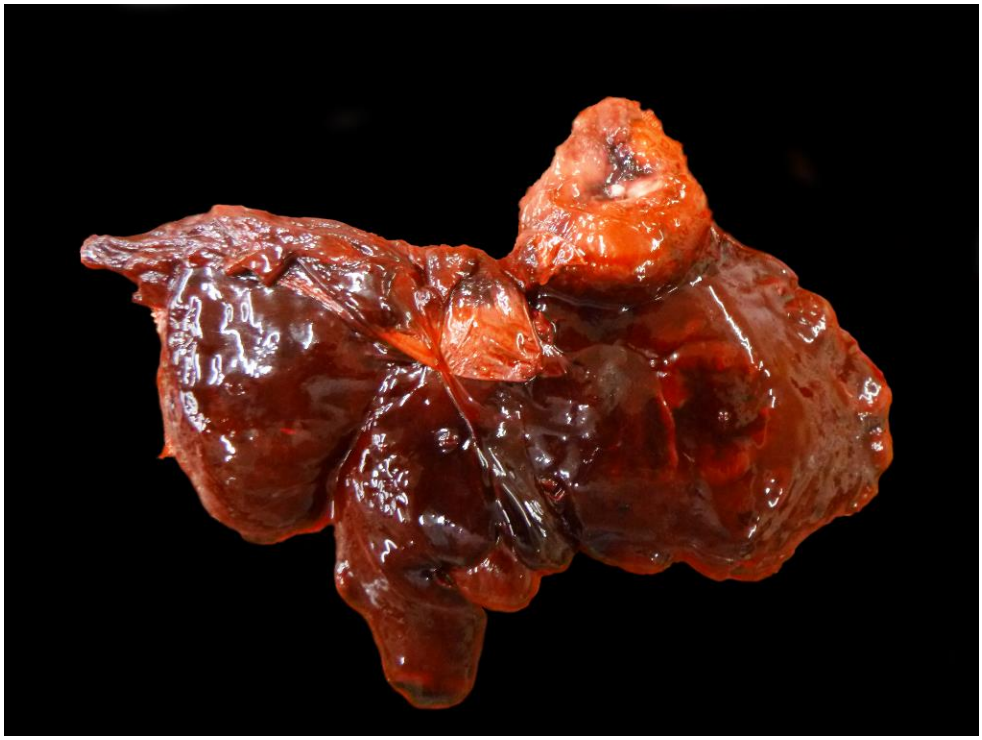

Figure 1. Liver (diaphragmatic aspect): the left lateral hepatic lobe is multifocally expanded and replaced by a firm, off white, nodule with a central indented brown-red area.

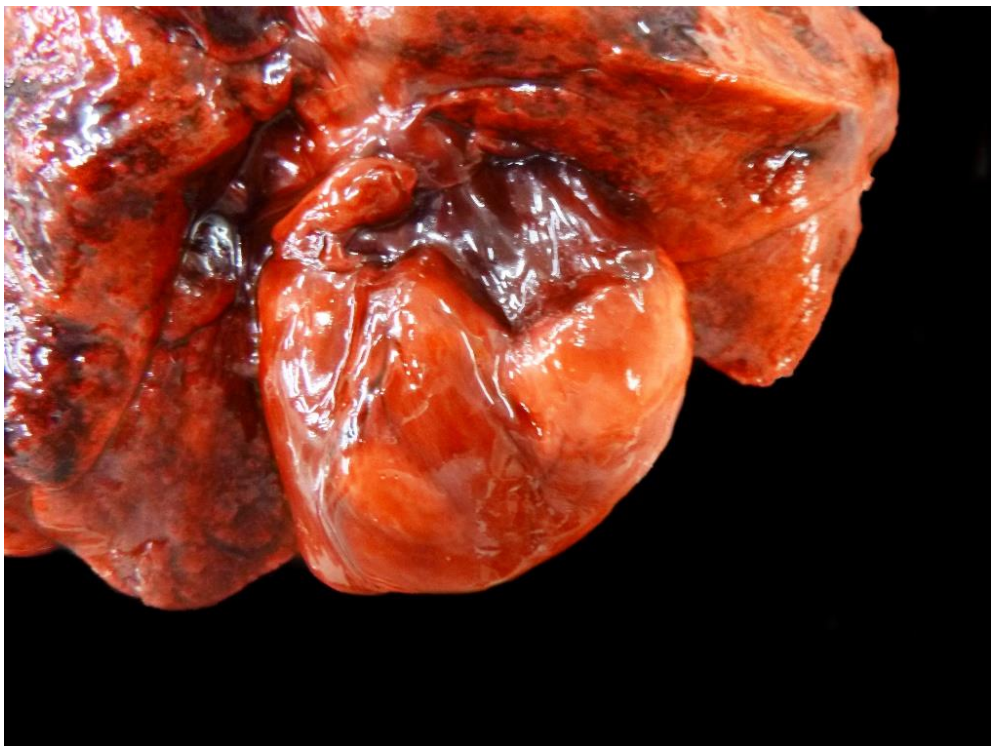

Figure 2. Lungs and heart: the right atrioventricular wall is partially replaced by multifocal firm, off-white nodular masses.

Microscopic pictures:

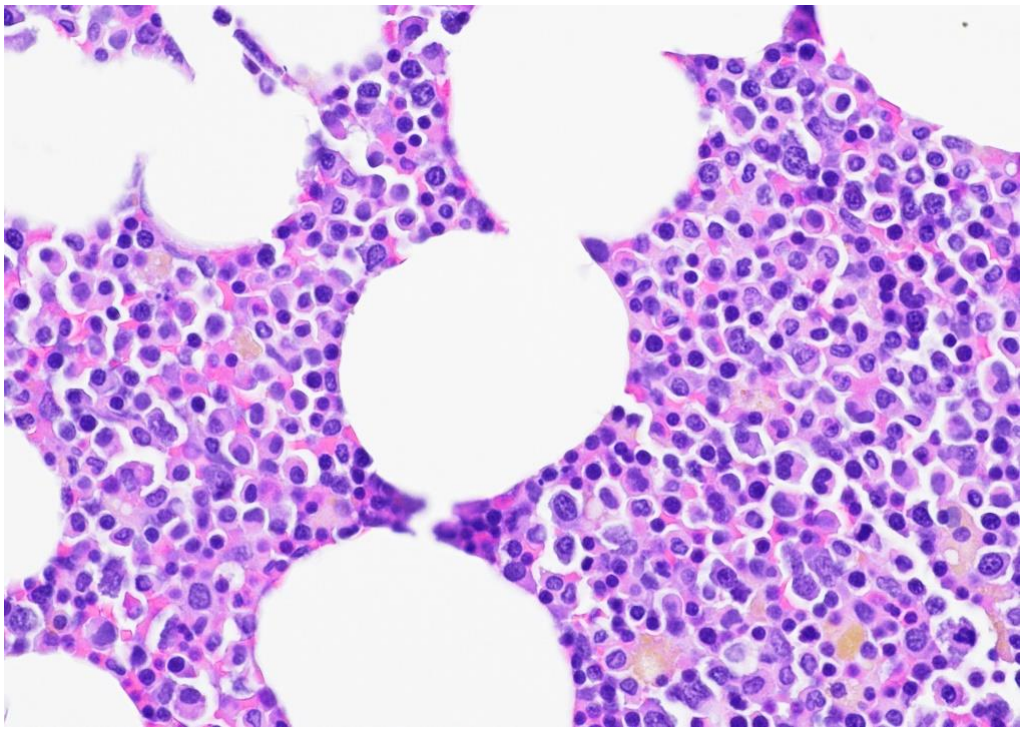

Figure 3. Bone Marrow, H&E, 40x: there is bone marrow infiltration by a highly cellular and poorly demarcated neoplasm. Neoplastic cells are round and have distinct cell borders, with an eosinophilic to amphophilic cytoplasm and occasional peri-nuclear halo; nuclei are round, oval or occasionally reniform, eccentric, with a finely stippled to finely clumped chromatin. There is moderate pleomorphism, anisocytosis and anisokaryosis.

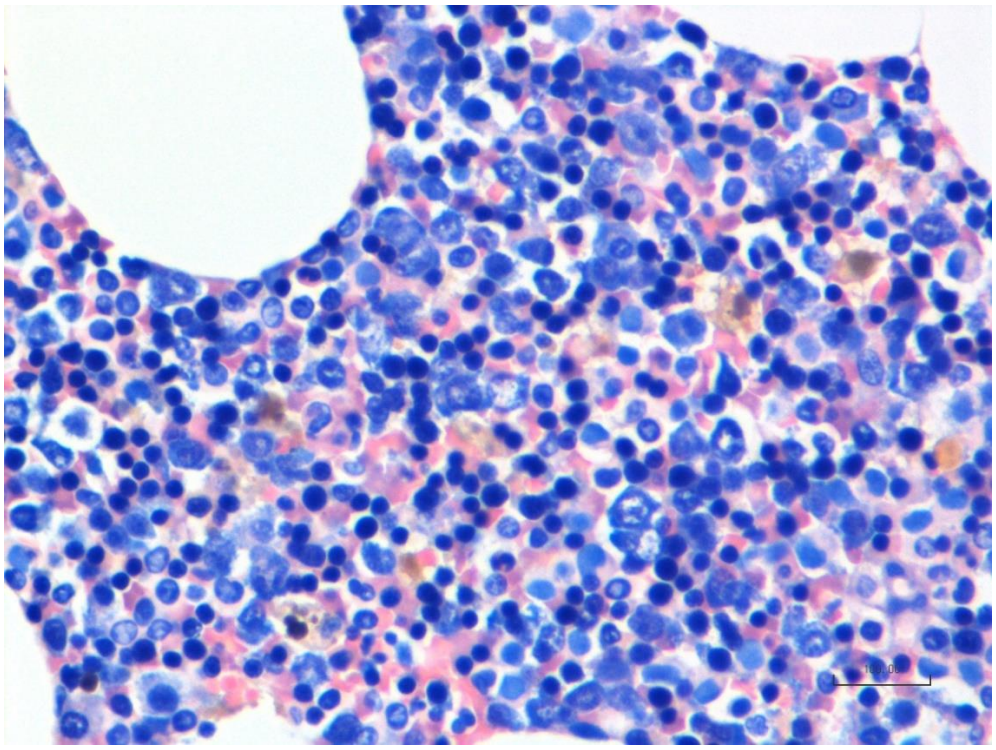

Figure 4. Bone marrow, Giemsa, 60X: cellularity is increased. Mature plasma cells are prominent, however there is a population of larger, round cells of uncertain origin, either of plasma cell origin or related to the erythroid line.

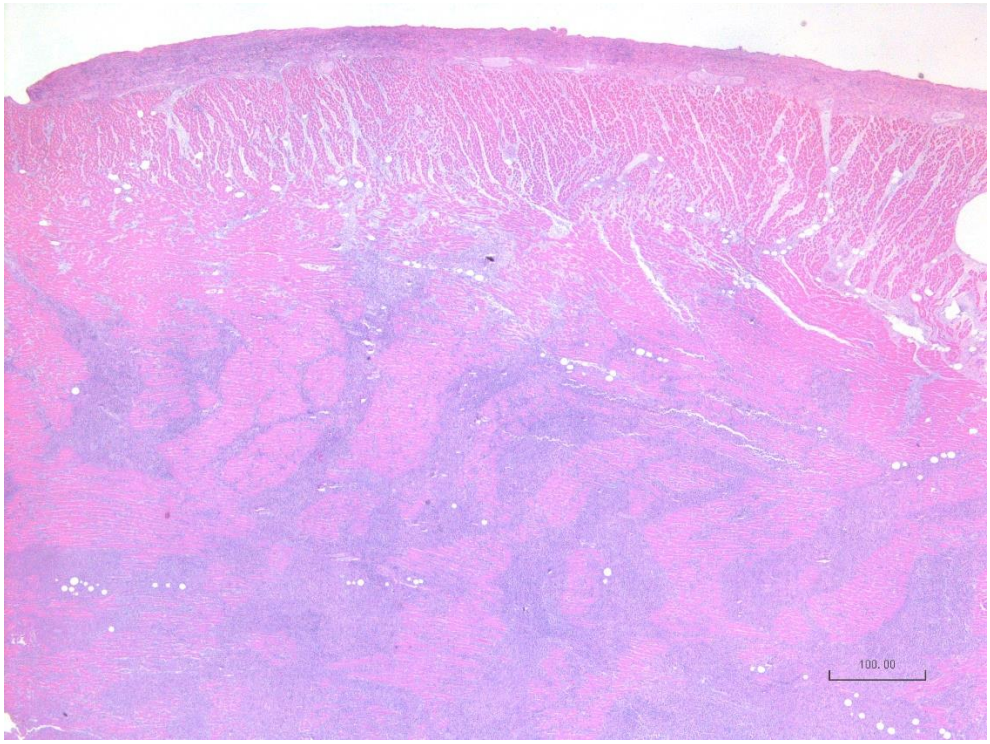

Figure 5. Heart, H&E, 2X: the right ventricular myocardium is multifocally infiltrated by neoplastic cells.

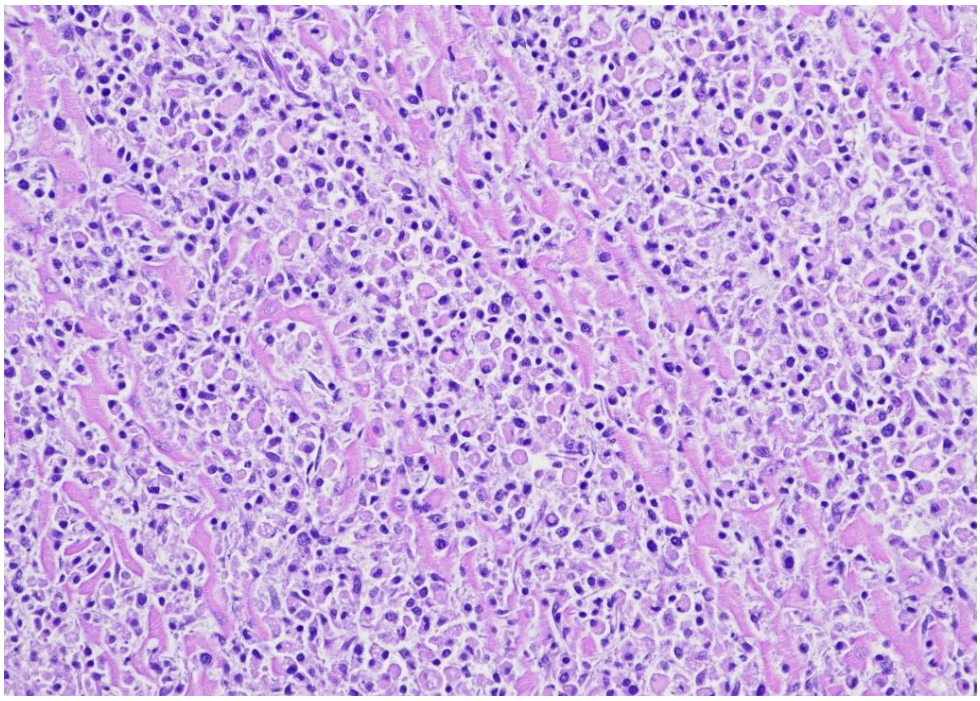

Figure 6. Heart, H&E, 20X: infiltrating the interstitium and individualizing cardiomyocytes, there is a neoplastic population of round cells with morphology similar to the neoplastic cells observed in the bone marrow.

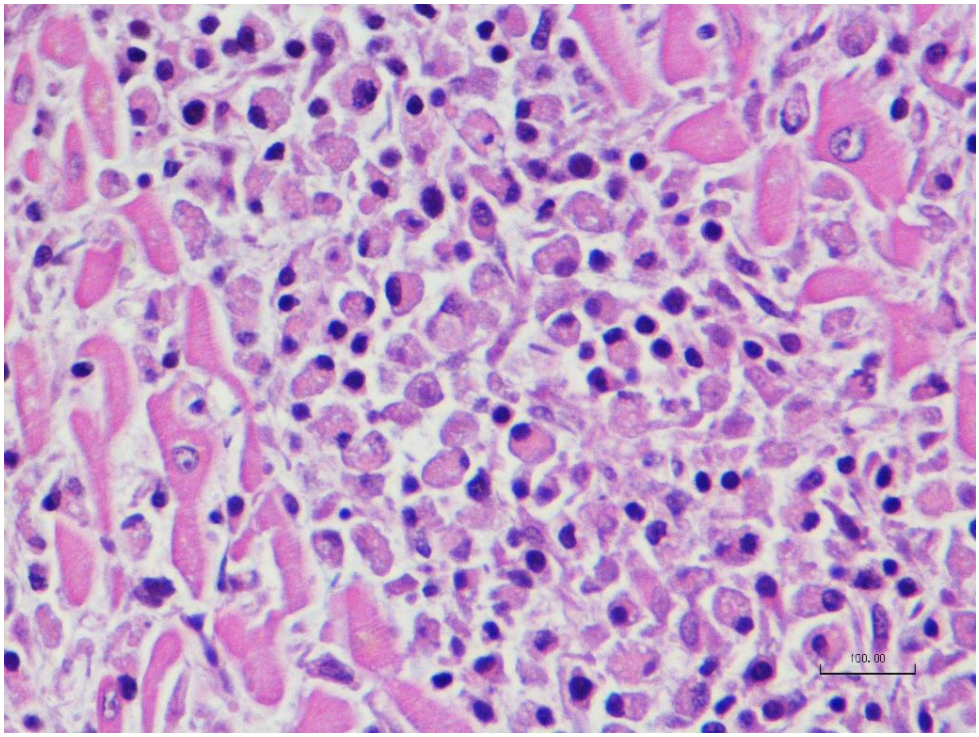

Figure 7. Heart, H&E, 60X: higher magnification of neoplastic cells with the majority having eccentric, round nuclei and ample eosinophilic to mildly amphophilic cytoplasm infiltrating the cardiac interstitium.

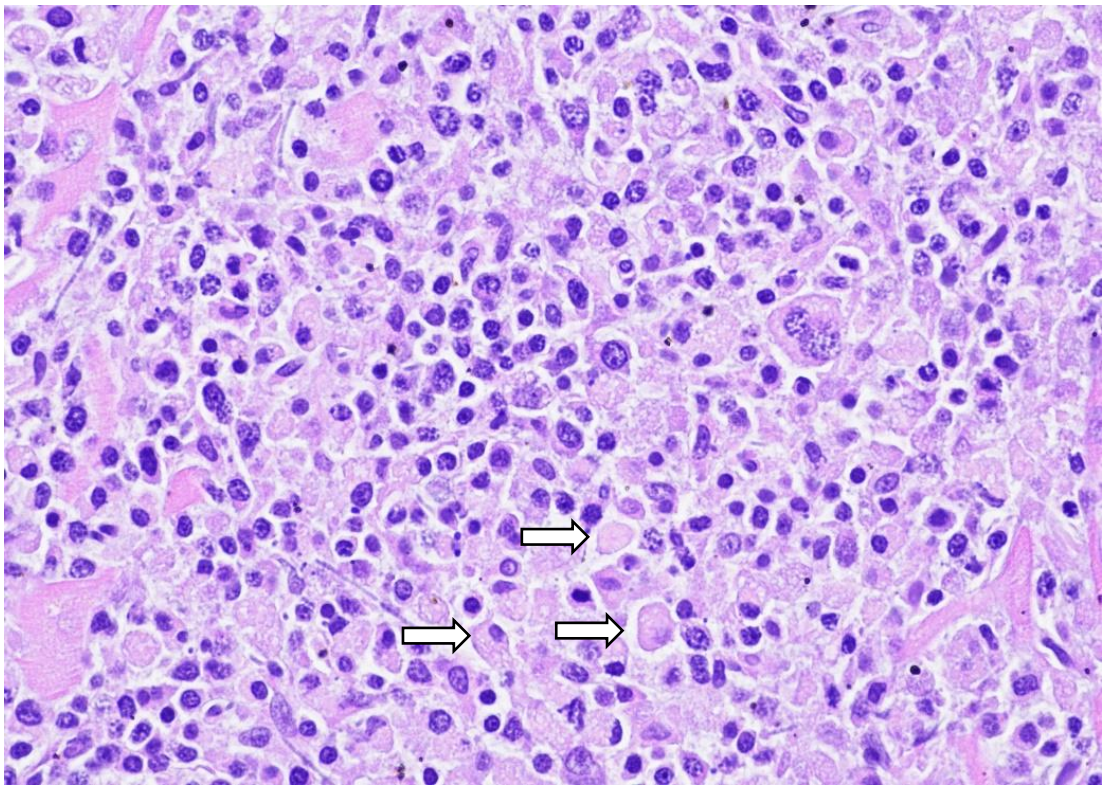

Figure 8. Heart, H&E, 40x: the neoplastic cells in this image are moderately pleomorphic compared to those in Figure 7, with moderate to marked anisocytosis and anisokaryosis. Some neoplastic cells have moderately large, nucleolated nuclei and small amounts of basophilic cytoplasm, whilst others are comparable to those in Figure 7. These latter cells are often filled with densely packed eosinophilic cytoplasmic vacuoles (possibly globulin-laden cells; white arrows). There is occasional binucleation of neoplastic cells.

Answers:

1. Gross descriptions:

Liver: The cranial aspect of the left lateral hepatic lobe is expanded by a raised, firm, off-white, sessile, circumferential mass with centrally depressed, highly friable, dark red focus (suggesting necrosis). Expanding the central aspect of the same hepatic lobe is a circumferential, slightly raised mass peripherally light red with an irregularly delineated dark red core.

Heart: Approximately 70% of the right atrioventricular wall is expanded and replaced by multifocal, raised, firm, off-white, nodular masses.

2. Gross morphologic diagnosis:

Liver (left and left lateral, medial lobes) and heart (right ventricular free wall): Metastatic neoplasia most likely.

3. Histopathological descriptions:

Bone marrow: Infiltrating and diminishing normal haemopoietic tissue, are multifocal, poorly demarcated small dense clusters of round cells with distinct borders (interstitial pattern). Anisocytosis and anisokaryosis are moderate to marked for the infiltrating cells. Many of these cells have an eosinophilic cytoplasm with possible droplet formation, and an eccentric round, ovoid or rarely reniform nuclei, with finely stippled to finely clumped chromatin. Cellular areas of bone marrow are about 60%, whilst adipose areas comprise 40%. Precursor erythroid cells are present, but erythropoietic islands are rare. A few metamyelocytes and band granulocytes are present. Scattered iron-laden macrophages are present.

Heart: Multifocally infiltrating and expanding the right ventricular myocardium are neoplastic cells with similar morphology as to those present in the bone marrow, forming thick bands filling the interstitium, often surrounding the epimysium and occasionally the perimysium individualising cardiomyocytes. Cells are moderately to markedly pleomorphic. There are myriads of neoplastic cells containing densely packed eosinophilic cytoplasmic vacuoles (possibly globulin-laden vacuoles), and occasional binucleation.

4. Histopathological morphologic diagnosis:

Bone marrow: Bone marrow: Infiltrating round cell neoplasm, suspected with plasmacytoid differentiation.

Heart: Metastatic round cell neoplasm suspected with plasmacytoid differentiation.

Conclusion: Metastatic round cell neoplasm, most likely lymphoproliferative in nature with plasmacytoid differentiation. Consider lymphoma with plasmacytoid differentiation or multiple myeloma.

5. Three (3) ancillary tests to confirm diagnosis:

To help confirm and delineate between lymphoproliferative disorders involving plasma cell differentiation, immunohistochemistry (1), and prior to death, haematology (2) and biochemistry (3) are useful ancillary tests. Immunohistochemistry (1) could be done on the bone marrow or heart sections (preferably both) for T and B lymphocytes (CD3, CD20, CD79a and PAX-5) and plasma cells markers (CD20, CD79a, MUM-1). Haematology (2) would assist in identifying a leukaemic manifestation and penias whilst blood biochemistry (3) could help identify dysproteinemias (especially of globulins) and organ tissue disease. For a definite diagnosis of multiple myeloma, and to differentiate it from malignant lymphoma with plasma cell differentiation, at least three criteria would need to be fulfilled: leukaemia, hyperglobulinaemia as well as two or more supportive paraneoplastic

syndrome findings such as hypercalcaemia, platelet dysfunction, hyperviscosity syndrome, cytopaenias and renal disease with Bence-Jones protein.

Discussion

Despite koalas and kangaroos usually sharing the podium for iconic Australian native and unique animals, dingoes (*Canis familiaris*) are Australia's only native canid, representing an important figure in Aboriginal and Torres Strait Islander communities' culture and spiritual practices. Their phenotype is similar of a domestic dog, but caution and respect must be practiced as they are wild. Currently, due to interbreeding with domestic dogs, dingo's genetic uniqueness is becoming threatened, with few known populations still isolated from interbreeding threats, such as the population of K'gari, Fraser Island, making conservation efforts for these groups important (3).

Cancer is a rising cause of morbidity and mortality through multiple species, including wildlife species that are currently endangered. Literature about neoplasms affecting dingoes is scarce, with few cases compiled in the Taronga Zoo's Australian Registry of Wildlife Health archive, which includes lymphoma, potential thymoma, lipoma, fibromatous epulis, perianal adenoma, sebaceous adenoma and squamous cell carcinoma (7).

In this case, a metastatic round cell tumour is suspected as cause of death. The abnormal right to left ratio (1:2) in the heart is likely due to the chronic, progressive impaired right ventricular outflow tract leading to right ventricular volume overload and subsequent dilation. This condition probably culminated in severe right ventricular failure leading to death.

Given the microscopic findings, a round cell tumour suspected of being either malignant lymphoma with plasmacytoid differentiation or the specific clinical syndrome of multiple myeloma were considered as the main differential diagnoses. Unfortunately – as is often the case with wildlife – radiologic, haematologic and blood biochemical testing was not done because of circumstances and budget restrictions. For similar reasons immunohistochemistry, although considered, was not performed.

Waldenström's macroglobulinemia is a subtype of lymphoplasmacytic lymphoma, a low-grade B-cell lymphoma, with infiltration of the bone marrow (BM) by small lymphocytes showing plasmacytoid or plasma cell differentiation and development of IgM monoclonal gammopathy. Due to infiltration of bone marrow, multiple variable cytopenia can be detected. In humans, bleeding disorders can be present, in association with a hyperviscosity syndrome. This disorder is rarely reported in animals and it's usually a diagnosis of exclusion, considering the lack of differentiation methods from other B-cell lymphomas based on morphologic, immunophenotypic and chromosomal changes. Immunophenotype features that support a diagnosis are positive labeling for IgM, CD19, CD20, CD22, CD25, CD27, FMC7, CD5 and plasma cells negative for CD138. Protein electrophoresis and immunofixation are needed to assess paraprotein (6).

Plasmablastic lymphoma (PBL) is a rare aggressive lymphoproliferative disorder characterized by monomorphic proliferation of round to oval cells with abundant cytoplasm, sometimes with perinuclear halo, and eccentric nuclei. Immunohistochemistry markers are of valuable help or the differentiation, with positive staining for MUM-1, CD138 and CD38 and negative staining for T and B cell markers such as CD3, CD20, and PAX5. Clinically, PBL has been characterized for its predilection of involving the oral cavity of HIV-positive or immunosuppressed humans, rapidly progressing and involving other organs (2,5). Multiple sites can be affected such as lymph nodes, skin, gastrointestinal tract, bone, and musculature. Giuliano et al (2023) reported a case suspected of PBL in a dog with clinical and anatomical topography similar to what is reported in humans. The immunohistochemistry staining was similar, MUM-1 positive and CD20, CD3 and PAX5 negative, except for CD79a, which was scantly positive for the dog. Additional markers for melanoma (SOX-1), histiocytic sarcoma (Iba-1) and mast cell tumour (CD117) were also negative. The animal lacked osteolytic lesions. Haematology and biochemistry were unremarkable, cytopenia, hypercalcemia and hyperglobulinemia were not present. The differentiation between plasmablastic multiple myeloma and plasmablastic lymphoma can be difficult due to identical morphological and immunohistochemical profile (5), therefore other factors such as clinical presentation -affected anatomic structures, disease progression, haematological and biochemical profile- are important to consider as well as further advance in molecular/genetic characterization in veterinary medicine.

Multiple myeloma (MM) is a primary tumour of bone characterized by malignant proliferation of terminally differentiated B lymphocytes. It is uncommonly diagnosed in animals, with domestic dogs being the most reportedly affected. Multiple bones are affected, mainly those active in haematopoiesis, presenting concomitant discrete lytic lesions. Clinical signs can vary depending on the affected site and include weight loss, lethargy, lameness, pain, paraplegia (when affecting vertebral bodies and injuring the spinal cord) (8). Generally, the diagnosis can be achieved by finding a minimum of two or three of the following changes: marked bone marrow plasmacytosis, monoclonal gammopathy, evidence of osteolysis, and Bence Jones proteinuria. Concomitant paraneoplastic syndrome indicators, such as hypercalcemia (resulting from the overproduction of osteoclastic-activating factors - RANKL, which leads to bone resorption), platelet dysfunction (arising from the binding of Ig/paraprotein to platelets, causing hemorrhages), hyperviscosity syndrome (attributed to increased blood viscosity due to IgM and IgA dimers, resulting in tissue ischemia and hemorrhages), cytopenias (resulting from the displacement of normal bone marrow), and renal impairment (stemming from hypercalcemia, hypoxic damage due to hemorrhages and/or hyperviscosity, and renal amyloidosis) can provide valuable clarification (1,8). When assessing the bone marrow, it has been proposed that a population of 30% or more of plasma cells should constitute the nucleated population to consider a multiple myeloma (1). In human medicine literature, the neoplastic cells can be classified as mature,

immature, pleomorphic, and plasmablastic, with three patterns of bone marrow infiltration: nodular, interstitial, and diffuse. Regarding morphology, mature cells won't display discernible differences from normal plasma cells, featuring a circular eccentric nucleus lacking nucleoli, abundant basophilic cytoplasm with a perinuclear clear halo. Immature myeloma cells exhibit an increased N:C ratio, with an irregular nucleus, scattered chromatin and visible nucleoli. Pleomorphic cells will display higher nuclear polymorphism, lobated nucleus, with prominent nucleoli. Plasmablastic cells are increased in size, with high nuclear polymorphism, and presence of mitotic figures, resembling diffuse large B-cell lymphoma. Dutcher bodies and Russel bodies are frequently identified in myeloma cells, the first highly specific of neoplastic process, whilst Russel bodies are grape-like accumulations seen in Mott cells and can be part of reactive processes. As for patterns of infiltration of the bone marrow, hematopoietic cells and adipose tissue are displaced by the neoplastic plasma cells, forming a distinctive nodule in the nodular pattern, whilst the interstitial pattern is characterized by compact clustering of neoplastic cells that infiltrate the spaces between the normal bone marrow tissue (4). Metastasis to other organs is most seen in the spleen, liver, lymph nodes, and kidneys (1). In Australian wildlife, myeloma is uncommonly documented, one report in the literature was found of a probable myeloma in a stranded bottlenose dolphin in Tasmania (7).

This case demonstrates the complexity of diagnosis of lymphoproliferative diseases showing plasmacytoid differentiation, especially without the support of ancillary testing. Although genetically close to domestic dogs, clinical manifestations of lymphoproliferative disorders are to date poorly studied in dingoes.

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The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation (DTF). These exercises are contributed by members and non-members from any country of residence. Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the DTF website (https://davisthompsonfoundation.org/diagnostic-exercise/).

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