ESVCP/ECVCP Mystery Case

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VIRTUAL SLIDE: A link to the virtual slide for this case can be provided to conference participants.

SPECIMEN: Pericardial fluid; direct smear

SIGNALMENT: 13 y/o MC Domestic Shorthair

HISTORY AND CLINICAL FINDINGS: One month history of hyporexia to anorexia; recent diarrhea. No significant findings on bloodwork. Has been treated for hyperthyroidism for nearly three years.

LABORATORY DATA:

Pericardial Fluid Analysis:

Fluid Color: Red
Supernatant Color: Orange
Fluid Clarity: Opaque
Supernatant Clarity: Clear
Nucleated Cell Count: 33,800/µl
Red Blood Cells: 350,000/µl
Refractometer protein estimate: 6.9 g/dl
Fluid hematocrit: 13%

Fluid total protein: 6.8 g/dl
Fluid albumin: 3.2 g/dl
Calculated fluid globulins: 3.6 g/dl
Calculated A:G ratio: 0.89

Images from the direct smear below:

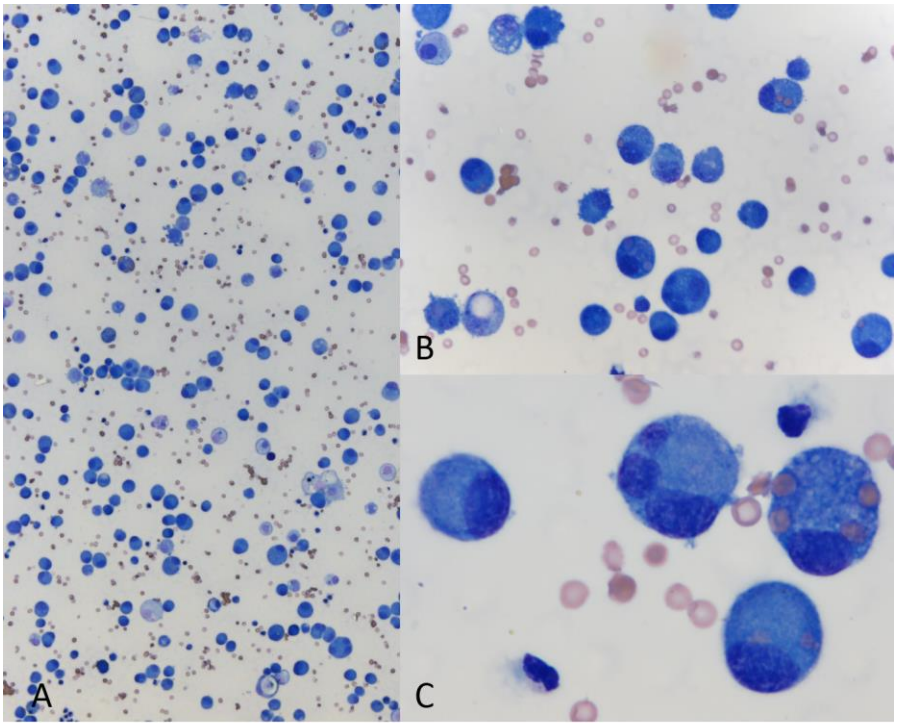

Figure 1. Pericardial fluid from a cat. Wright-Giemsa stain.
A) Magnification = 100x
B) Magnification = 500x
C) Magnification = 1000x

ADDITIONAL DIAGNOSTIC TESTS:

Multiplexed fluorescent immunocytochemistry for pancytokeratin and vimentin was performed on cytocentrifuged samples of this pericardial fluid.

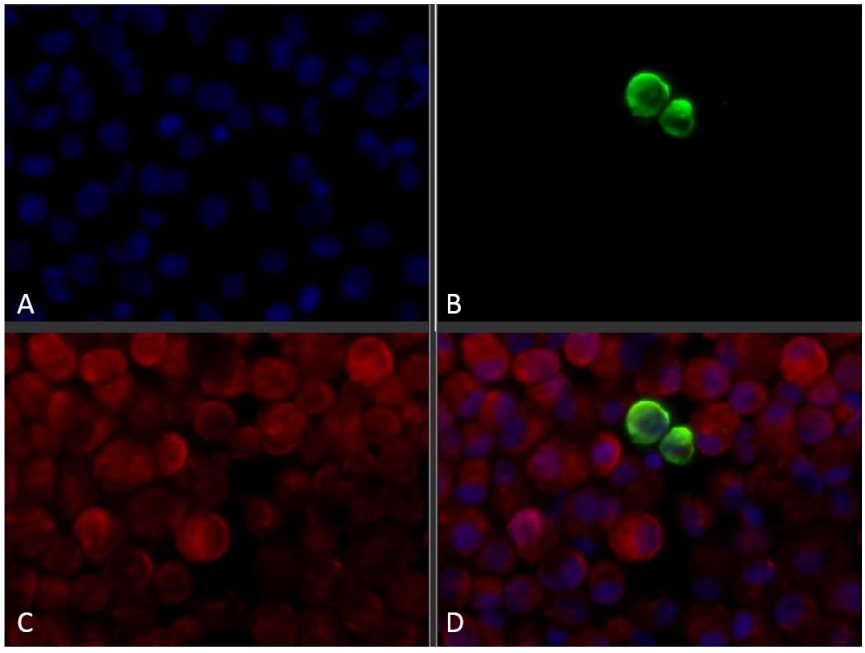

Figure 2. Multiplexed fluorescent immunocytochemistry. Magnification = 400x. Isotype negative control stained appropriately (not shown).
A) Cell nuclei (DAPI, blue)
B) Cytokeratin (FITC, green)
C) Vimentin (Alexa 594, red)
D) Overlay

QUESTIONS:

1. What are your top differentials for these cells type based on morphology?

2. Do the results of fluorescent multiplex immunocytochemistry help narrow down the list of differentials?

3. What additional diagnostic tests on this fluid would you recommend?

CYTOLOGIC DESCRIPTION:
The sample is highly cellular and consistent with the count obtained. A pale basophilic background indicates proteinaceous fluid, consistent with the high protein level reported. Predominant cells are large, discrete, round cells with typically a single but occasionally double or multiple nuclei. Nuclei are often eccentrically located, chromatin is stippled and there is often a single prominent nucleolus. The cytoplasm is moderately abundant and moderate to deeply basophilic. Occasional mitotic figures are observed. Approximately 10-20% of the cells contain phagocytized erythrocytes and occasional cells are vacuolated. The cells exhibit moderate to marked anisokaryosis with variably sized satellite nuclei and moderate anisocytosis. There are lesser numbers of intact and degenerate neutrophils and macrophages. Some of the macrophages are also erythrophagocytic, indicating recent hemorrhage.

Based on multiplex fluorescent immunocytochemistry (MF-ICC), the majority of the cells express vimentin and do not express cytokeratin, indicating that the majority are of mesenchymal and not mesothelial origin. Occasional double-positive cells are present among the neoplastic cells (see Figure 2), consistent with few reactive mesothelial cells. Alkaline phosphatase staining is negative (not shown), making osteoblastic origin highly unlikely.

CYTOLOGIC INTERPRETATION/DIAGNOSIS: DISCRETE CELL NEOPLASIA
This tumor has cytologic features of both plasma cells and histiocytic cells. Atypical lymphoma cannot be entirely ruled out.

ADDITIONAL FINDINGS:

Ultrasound findings:

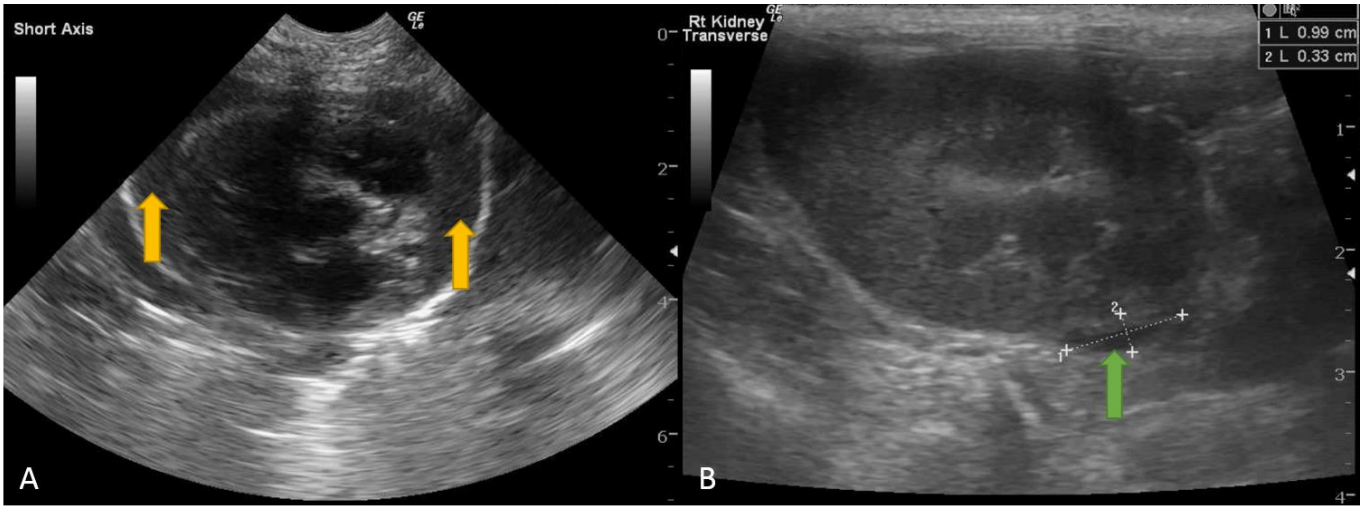

Figure 3. A) Echocardiogram, short axis. There is a moderate amount of echogenic pericardial fluid (yellow arrows). This finding is unusual in cats, and it is suggestive of a cellular effusion. Neoplasia is considered most likely; pericarditis is also possible. B) Right kidney. Two hypoechoic regions bulged from the cortical margins (one shown here, green arrow). Infiltrative neoplasia is considered most likely for these lesions.

Patchy areas of increased renal cortical echogenicity in the left kidney, and slight wall thickening of the distal ileum (not shown) were also consistent with potential infiltrative disease.
Special stains (chromogenic immunocytochemistry) were performed on direct smears of the pericardial fluid:

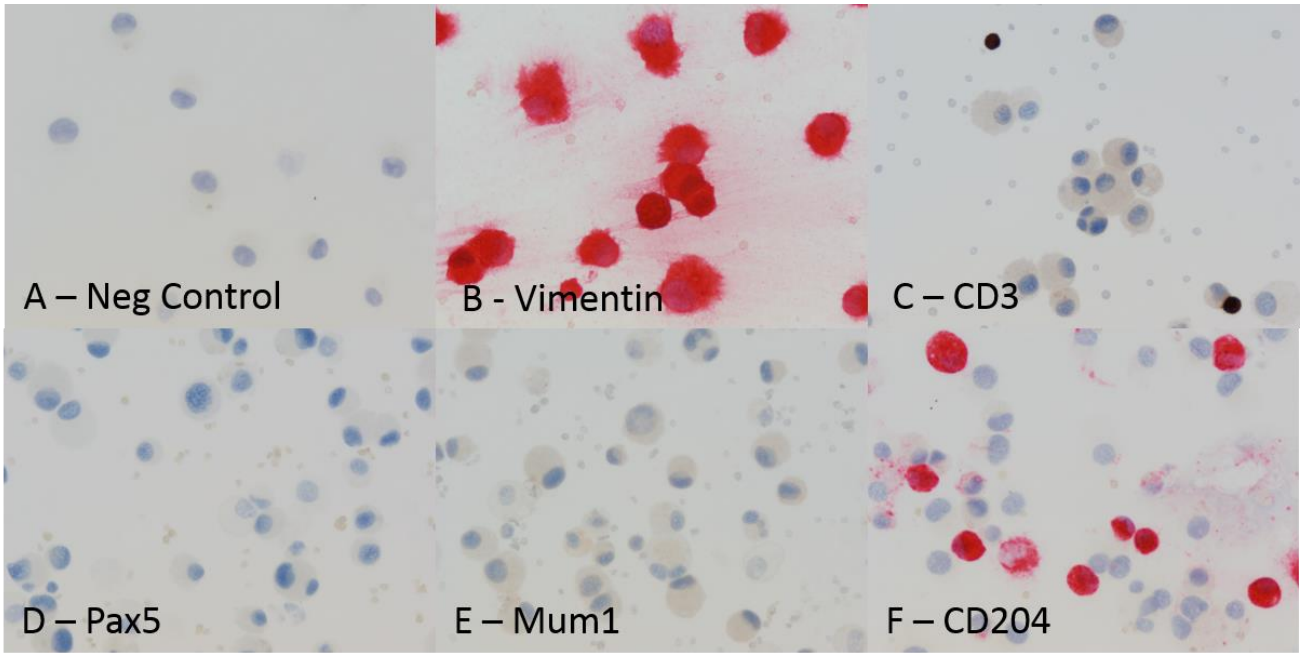

Figure 4. Chromogenic immunocytochemistry; magnification = 500x. A) Negative isotype control; B) vimentin (mesenchymal marker), C) CD3 (T-lymphocyte marker); D) Pax 5 (B-lymphocyte marker); E) Mum-1 (plasma cell marker); F) CD204 (macrophage marker).

Cells in this sample were strongly immunoreactive to vimentin (confirming this result from MF-ICC) and negative for canine CD18 (not shown), Pax5, and Mum-1. Few small, CD3-positive cells were admixed with the neoplastic population and interpreted as normal infiltrating T-lymphocytes (Figure 4C). Occasional cells (~30%) were strongly positive for the histiocytic marker CD204[1], including some of the cells with multiple or bizarre nuclei (Figure 4F).

PCR for antigen receptor rearrangement (PARR) was performed on a previously-stained cytology slide. The PARR assay was potentially suggestive of the presence of a clonally rearranged immunoglobulin gene, but the presence of oligoclonal peaks and concerns of low cellularity prevented the sample from being definitively positive. A secondary PARR assay designed to detect partial rearrangements and clonally rearranged immunoglobulin light chain genes in cats was negative.

DIAGNOSIS: DISCRETE CELL TUMOR; PROBABLE HISTIOCYTIC ORIGIN

Positive immunoreactivity for CD204, along with cytomorphology, erythrophagia, MF-ICC, and negative PARR results, led to a cytologic and clinical diagnosis of probable hemophagocytic histiocytic sarcoma. Though an anaplastic plasma cell tumor cannot be entirely ruled out (plasma cell tumors have been reported to be erythrophagocytic in both dogs and cats[2–4]), it is

considered unlikely based on lack of Mum-1 staining and lack of definitively positive PARR results. Though typical splenomegaly and other organomegally (e.g.; liver, lymph nodes) were not present in this case, there were suspect infiltrative lesions in the left ventricle, left and right kidneys, and distal ileum. Thus the constellation of findings is most consistent with the insidious, infiltrative (non mass-forming) nature of hemophagocytic histiocytic sarcoma in cats[5,6], which is supported by the prominent erythrophagia observed by cytology.

CLINICAL OUTCOME/FOLLOW-UP: This patient was subsequently euthanized for poor quality of life, and necropsy was not performed.

ANSWERS TO QUESTIONS:

1. What are your top differentials for these cells type based on morphology?

Plasma cells or histiocytes. Less likely, atypical mesothelial cells or lymphocytes.

2. Do the results of fluorescent multiplex immunocytochemistry help narrow down the list of differentials?

Yes, mesothelial origin is no longer a consideration.

3. What additional diagnostic tests on this fluid would you recommend?

Immunocytochemistry for CD3, Pax5, Mum-1, and CD204, and/or flow cytometry for typical hematopoietic markers. PARR testing is also recommended to help rule in or out plasma cell tumor or lymphoma.

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