A jejunal mass in a 2-year-old dog

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Case Presentation: A two-year-old, spayed female mixed breed dog presented to the Purdue University Veterinary Teaching Hospital Small Animal Internal Medicine Service for chronic regurgitation and diarrhea with intermittent vomiting, which had been waxing and waning for approximately six months. Endoscopy of the gastrointestinal tract revealed no significant abnormalities, and endoscopic biopsies returned as lymphoplasmacytic enteritis. An abdominal ultrasound revealed a small 1cm x 1 cm x 1 cm mass on the serosal aspect of the jejunum; however, the owners declined further evaluation at this time. She was initially prescribed Cisapride and Omeprazole and was monitored at home.

The patient presented again for a recheck two weeks later, at which point her clinical signs had resolved. Initial physical exam findings were unremarkable except for an episode of coughing during the exam. The owners noted that the patient was developing a cough at home as well. CBC findings were within normal limits except for a mild leukopenia (5.8 k/µL [6-17 k/µL]), with lymphocytes (1.2 k/µL [1-5 k/µL]) and monocytes (0.17 k/µL [0.15-1.35 k/µL]) at the lower end of the reference interval. A SNAP 4Dx Plus Test was negative. Thoracic and abdominal radiographs also revealed no significant findings. On abdominal ultrasound, the jejunal nodule was identified again and appeared static in size. Fine needle aspiration of the nodule was performed and submitted for cytologic evaluation (figures 1 and 2). The mass was later removed, and impression smears of the mass were performed with identical findings (figure 3).

Figure 1: FNA from jejunal mass (60x magnification, Modified Wright). Presence of abundant intermediate to large lymphocytes and Mott cells (arrows).

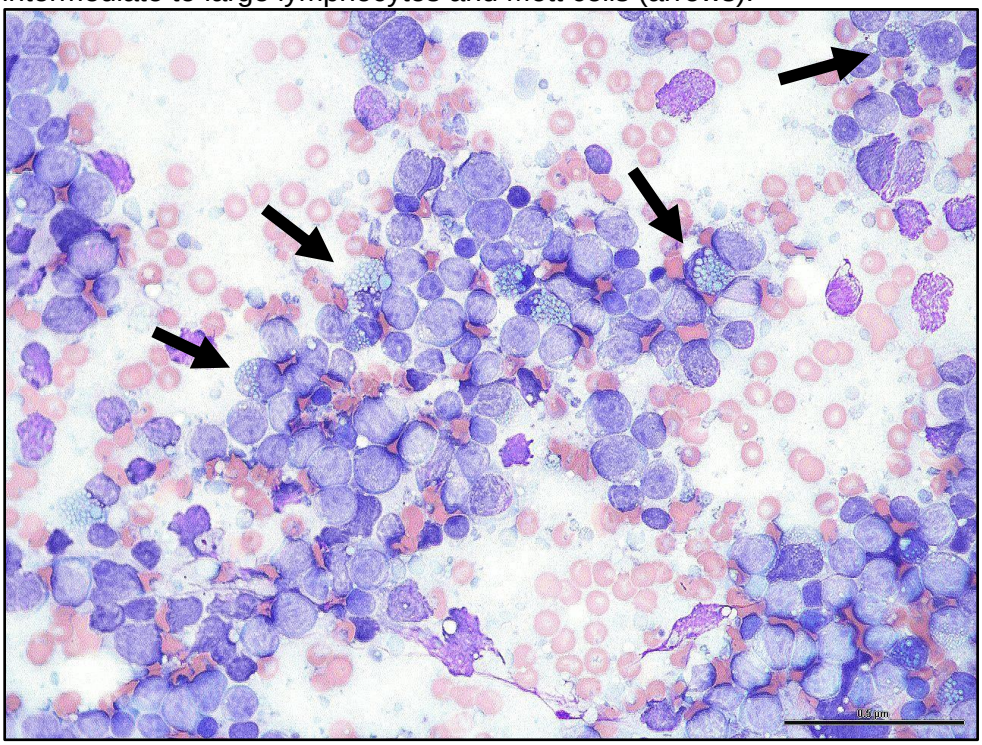


Figure 2: FNA from jejunal mass (100x magnification, Modified Wright).

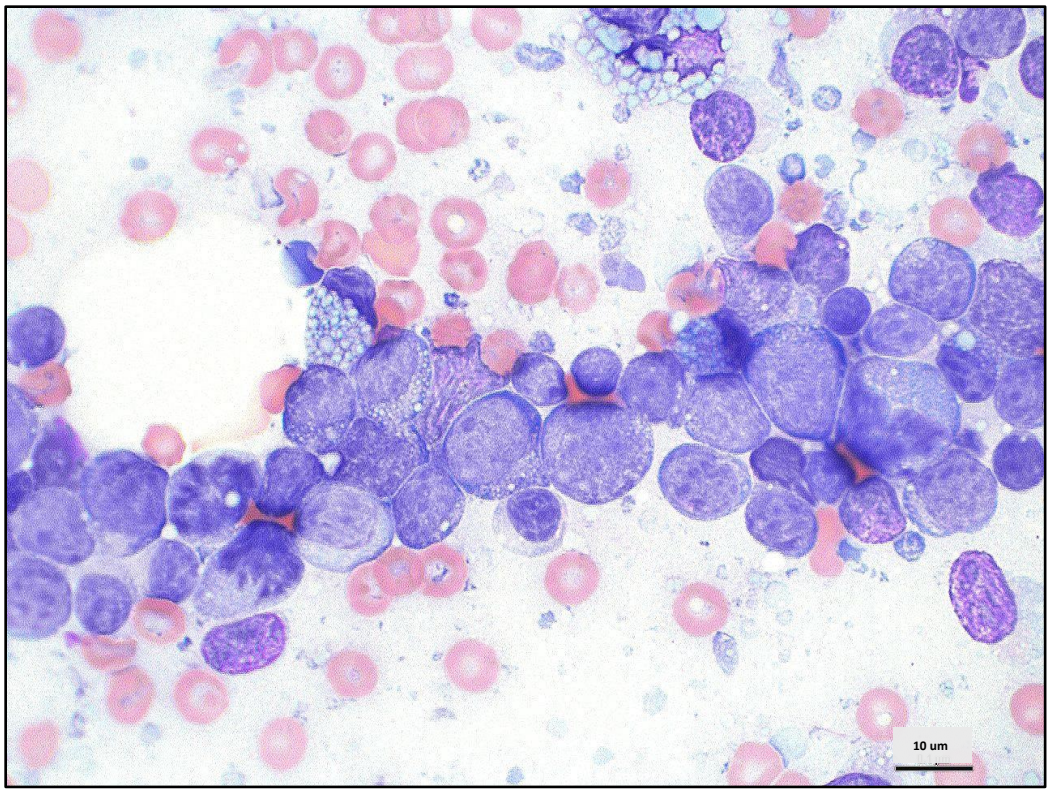
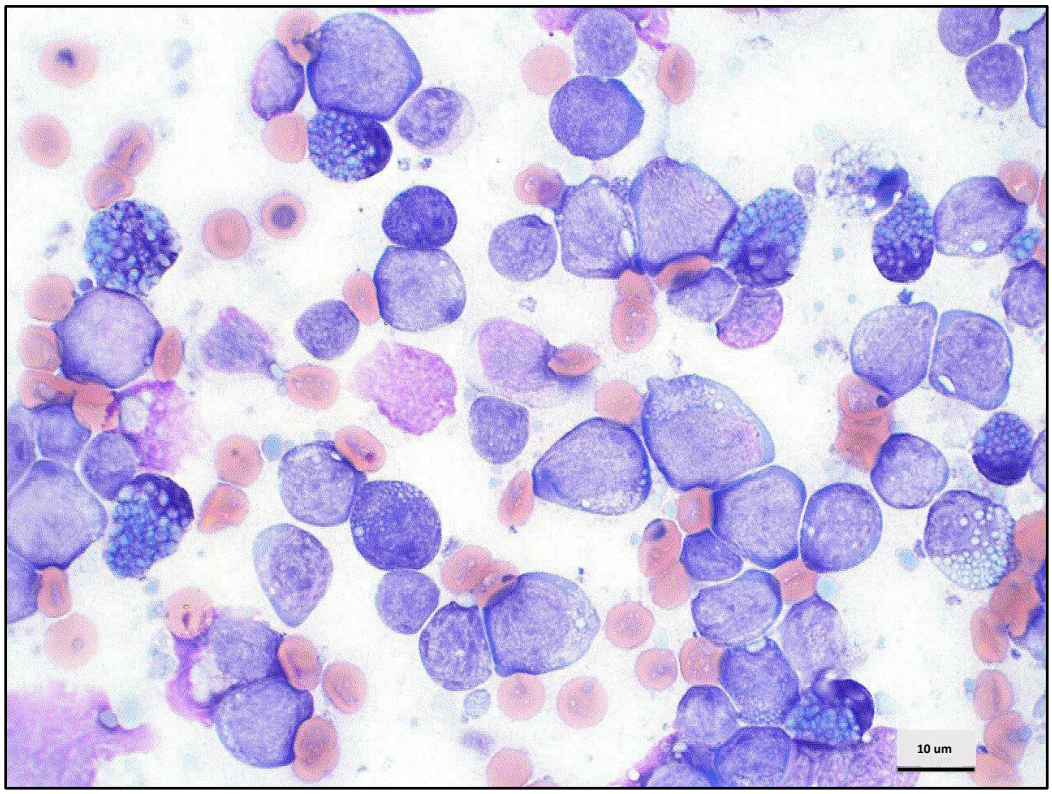


Figure 3: Impression smear from jejunal mass (100x magnification, Modified Wright).



Cytologic description of the fine needle aspirate/impression smears:

The smears show a marked expansion of intermediate to large lymphocytes measuring 12-20 µm in diameter (about 70% of the total lymphocyte population). They have a scant amount of dark blue cytoplasm that frequently contains variable numbers of light blue, round inclusions consistent with Russell bodies. Their nuclei are round to ovoid in shape with a finely stippled chromatin pattern and 1-3 prominent, round to irregular nucleoli. Also observed are numerous Mott cells measuring 12-15 um in diameter that comprise 40-50% of the nucleated cells in the aspirate preparations. They have abundant round, light blue inclusions that completely fill the cytoplasm and peripheralize the nucleus. Their nuclei are round with a clumped chromatin pattern. There appears to be a continuum between the large lymphocytes and the Mott cells with the absence of a characteristic plasma cell morphology. Rare small, mature lymphocytes are also scattered in the preparation. Nondegenerate neutrophils are consistent with blood contamination. No inflammation or infectious agents are identified.

Cytologic interpretation:

Lymphoma with Mott cell differentiation

Additional Diagnostic Tests:

PARR of the aspiration cytology: Samples of the aspiration cytology were submitted to the Colorado State University's Clinical Immunology Laboratory for PARR and revealed a clonal rearrangement of the B cell immunoglobulin gene and a polyclonal T cell population, further supporting a neoplastic B cell proliferation.

Due to the diagnosis of neoplasia, the owners elected surgical removal of the nodule, which was submitted for histopathological evaluation.

PARR of the FFPE tissue: FFPE tissue of the jejunal mass was submitted to the Colorado State University's Clinical Immunology Laboratory for PARR and was positive again for a clonal B cell population. It was noted that the primers used for the PARR on the aspiration cytology were different than the PARR used for the FFPE tissue.

Serum Protein Electrophoresis: Though the patient did not have a hyperglobulinemia (2.7 g/dL [1.7-3.8 g/dL]), serum protein electrophoresis was pursued to completely rule out a monoclonal gammopathy. Only a mild hypogammaglobulinemia was noted, most likely due to the young age of the patient.

Biopsy/Histopathology: In contrast to the cytology and PARR findings, the histopathological findings were most consistent with a markedly hyperplastic lymphoid follicle with retention of normal follicular architecture. The lymphocytes appeared to distend the muscularis layer of the jejunum. Marked numbers of Mott cells were also identified, confirmed by MUM1 and PAS staining. Further evaluation with markers CD3, CD20, and Pax5 were also performed (Fig 4-7). The follicles are lined by normal $CD3^+$ T cells. Pax5 showed nuclear positivity and CD20 showed membranous positivity within the immature B cells of the follicle. The differentiated Mott cells were Pax5 negative and often CD20 negative or faintly positive. Pax5 positivity was concentrated near the rim of T cells and in the center of the follicle. Interestingly, the Mott cells appear to be concentrated between the $Pax5^+$ $CD20^+$ B cells near the rim of T cells and the center of the follicle.

Figure 4: 4x magnification of H&E (A), CD3 (B), CD20 (C), MUM1 (D), and Pax5 (E) staining of a section of the jejunal nodule. (F) 60x magnification of PAS.

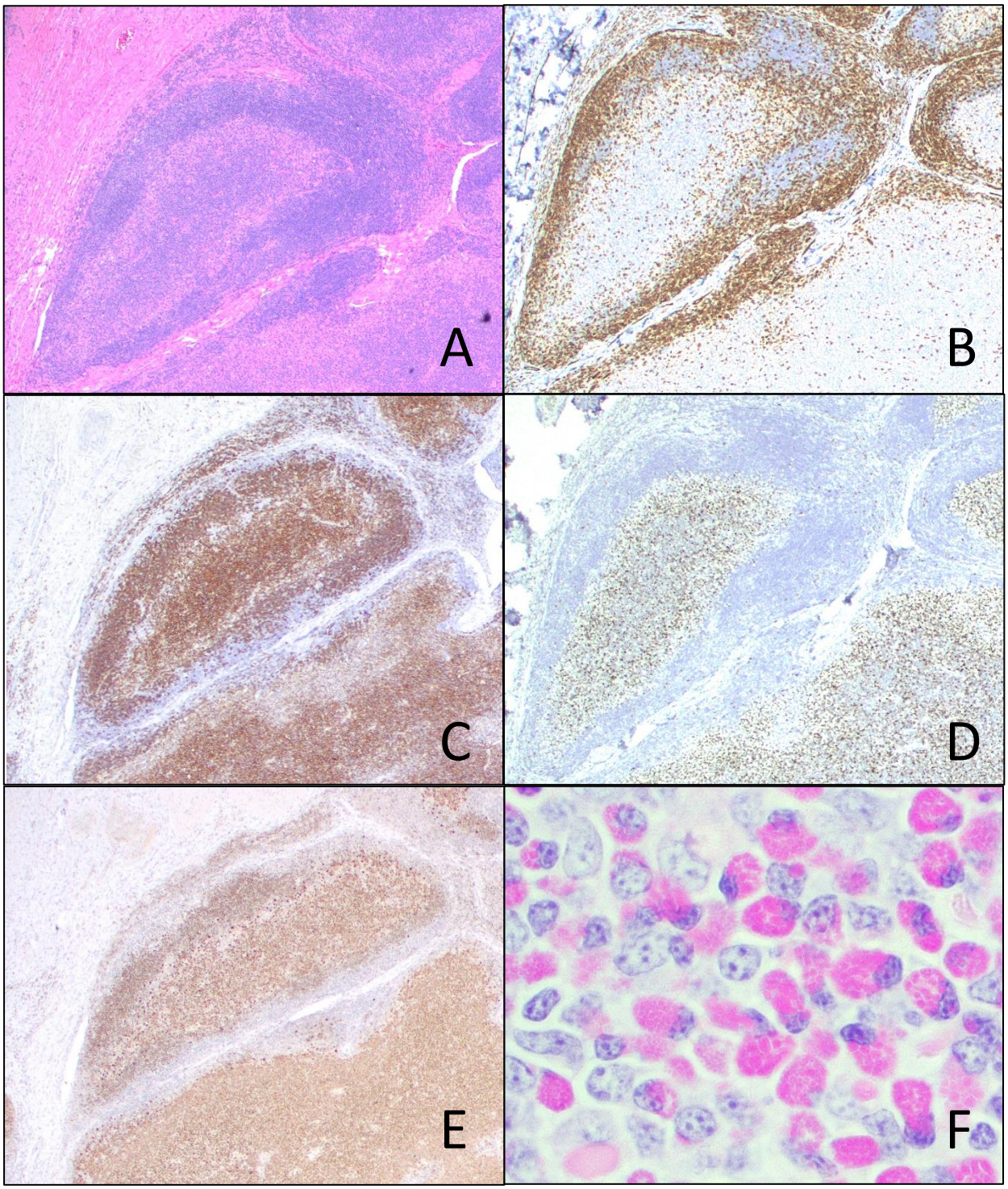


Figure 5: 60x magnification of Pax5 IHC of the jejunal mass, showing that the nuclei of the Mott cells are negative with non-specific cytoplasmic staining (arrows). Contrast to the positive immature B lymphocytes in the image (circle).

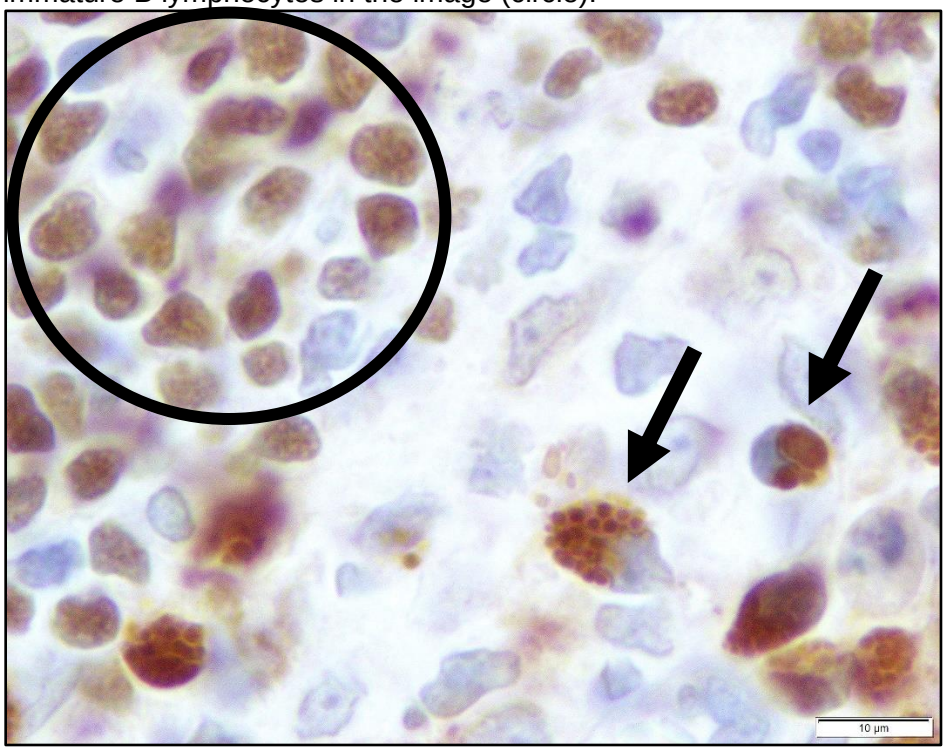


Figure 6: 4x magnification of CD20 IHC of jejunal mass. The immature B cells have positive membranous staining while Mott cells are weakly positive to negative. A band of Mott cells separate the immature B cells into an outer ring and a central core within the follicle.

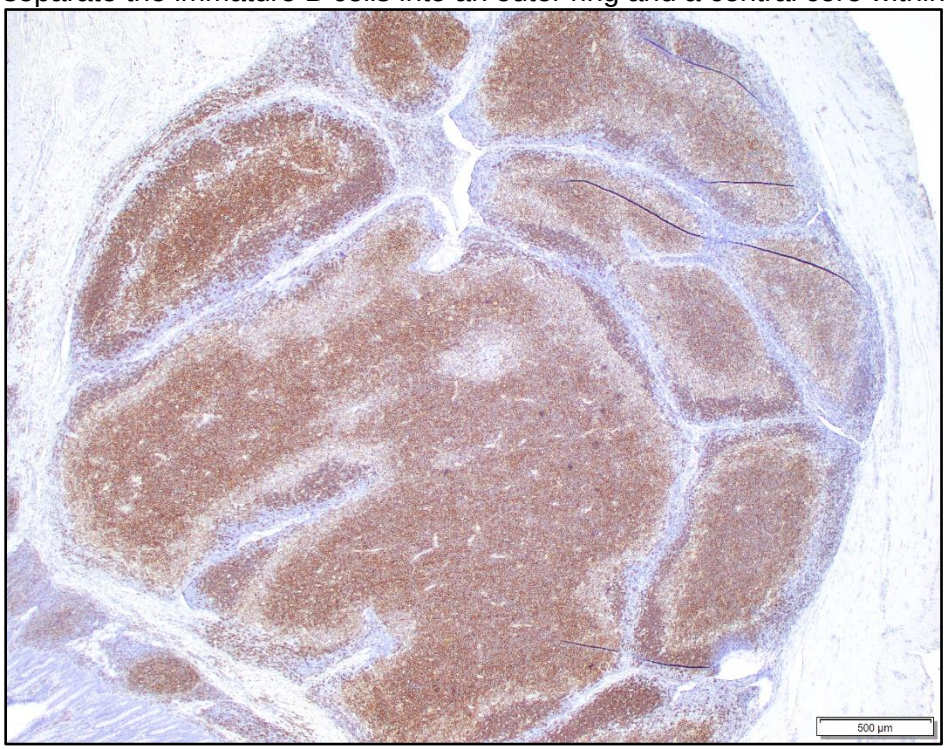
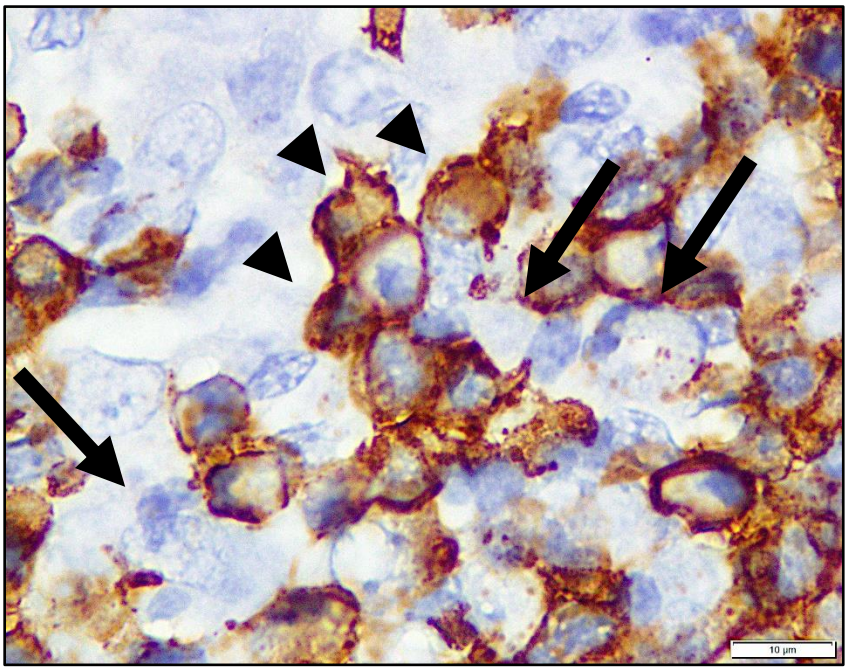


Figure 7: 100x magnification of CD20 IHC of jejunal mass. Greater resolution showing the negative staining of the Mott cells (arrowheads) in comparison to the immature lymphocytes (black arrows).



Patient outcome:
The owners are continuing to monitor the patient at home with no surgical complications. She continues to have no gastrointestinal signs. The patient is due back for a three month recheck for further follow up.

Discussion:
The monotonous large lymphocyte population observed in the aspiration cytology and impression smears were concerning for a neoplastic proliferation of B lymphocytes. Abundant numbers of Mott cells were also identified. Mott cells are terminally differentiated B lymphocytes with abundant cytoplasmic inclusions containing immunoglobulins (Russel bodies). Normally, B lymphocytes mature into plasma cells prior to adopting the Mott cell phenotype[1]. However, in this case, there was no evidence of plasmacytoid morphology in the aspiration cytology or histopathology. Moreover, there appeared to be a continuation between the large lymphocytes and the atypical Mott cells, suggesting that the immature lymphocytes may have matured directly into the Mott cells.

The expression profile of the Mott cells in this case was generally consistent with normal Mott cells and plasma cells (MUM1+, Pax5-, CD3-). Most of the Mott cells in the sample were also usually $CD20^-$, which is expected in normal or reactive populations. Faint membranous positivity for CD20 was occasionally suspected, however the marked amount of surrounding $CD20^+$ immature B-cells decreased confidence in this finding. CD20 positivity in atypical plasma cells (non-normal/reactive) has been observed in rare cases[2,3].

This finding was initially concerning for a B-cell lymphoma with Mott cell differentiation. This is a rare subtype of B-cell lymphoma, with few case reports in dogs[1,4-7], one cat[8], and one ferret[9]. These neoplasms were associated with the stomach or small intestine in all reported canine

cases, and often were associated with gastrointestinal signs including vomiting, diarrhea, and anorexia[1,4-7]. Too few case reports exist to determine the morbidity and mortality of the disease process in comparison to other B-cell lymphomas, and clinical outcomes ranged from rapid decline and euthanasia within 3 months[1] to complete remission with cyclophosphamide-vincristine-prednisone chemotherapy[6]. This case is unique in that there is a discordance between cytology and histopathology of the jejunal mass. While the cytologic findings were concerning for lymphoma with Mott cell differentiation, the histopathological findings were most consistent with a hyperplastic follicle.

PARR was performed on the cytologic samples as well as the FFPE tissue, and both returned with evidence of a monoclonal B cell proliferation. It is possible that this represents a false positive result in both PARR assays, which can occur with certain infectious agents such as Leishmania spp.[10], Ehrlichia spp., and Helicobacter pylori[11], though these differentials are not suspected in this case. The patient was negative on an IDEXX SNAP 4DX test, and no abnormalities were observed in the patient's stomach during endoscopy. Another possibility is the presence of an indolent lymphoma (such as a follicular lymphoma) that has not caused effacement of normal nodal architecture.

Another atypical finding was the unusual immunohistochemical staining pattern of the follicles. While all the follicles were lined by $CD3^{+}$ T cells, a band of mature $MUM1^{+}$, $CD20^{weak\ +/-}$, $Pax5^{-}$ Mott cells were seen separating an outer ring and an inner center of $CD20^{+}$, $Pax5^{+}$ immature B cells. The significance of this pattern is unknown; however, a possibility may be a neoplastic proliferation of B cells maturing into Mott cells and pushing the "normal" polyclonal B cells to the periphery. Currently, the final diagnosis remains inconclusive, and the patient remains under supervision for further follow-up.

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Question 1: Which immunohistochemistry marker is LEAST likely to be positive in plasma cells?

A. MUM1
B. CD79a
C. Pax5
D. CD45

Answer: C. Pax5

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Question 2: Which type of lymphoma is considered the feline equivalent to Hodgkin's disease?

A. Marginal zone lymphoma
B. T-cell-rich large B-cell lymphoma
C. Diffuse large B-cell lymphoma
D. Peripheral T-cell lymphoma

Answer: B. T-cell-rich large B-cell lymphoma

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