Mesenteric lymph node cytology in cats for diagnosing small cell granular lymphocyte intestinal lymphoma

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Abstract

Intestinal lymphoma is the most common lymphoma in cats, and granular lymphocyte (GL) type is a common variant. Mesenteric lymph nodes (mLNs) are often used as a surrogate sample when there is intestinal thickening. It is currently not known how many GLs are present in normal mLNs, and how many are indicative of underlying intestinal GL lymphoma. mLN aspirates were evaluated to quantify the % GLs present and to determine their molecular clonality status (clonal or reactive). When available, accompanying intestinal biopsies were also interrogated to confirm the pathologic diagnosis. Results from each cytology and biopsy were correlated and sorted into diagnostic categories. Results indicate that mLNs of cats with normal or inflamed intestine have < 1% small granular lymphocytes while those of cats with intestinal GL small cell lymphoma have 2.78-57.5% SGLs. These findings support our hypothesis and provide useful guidance for the diagnostic workup of cats with intestinal disease.

Introduction

Hematopoietic neoplasms account for approximately 30% of all tumors in cats, and among these, lymphoma is the most common. Intestinal lymphoma accounts for most, 32%-72%, of lymphomas diagnosed in cats. Most are T cell tumors and involve the small intestine. Numerous morphologic cell types occur in feline intestinal lymphoma. One of the most common is granular lymphocyte type, usually derived from resident intra-epithelial cytotoxic T cells. Intestinal lymphoma can be diagnosed cytologically by needle aspiration. As direct aspiration of thickened intestine may be problematic, clinicians often sample mesenteric lymph nodes as a surrogate tissue since they are the first site of distal metastasis. It is currently not known how many GLs are present in normal lymph nodes, and what threshold indicates the presence of underlying intestinal lymphoma. Correlation of GL % with TCRγ gene rearrangement molecular clonality PCR status will help determine what % of GLs is associated with underlying intestinal lymphoma and hence provide useful diagnostic guidelines.

Objective

To determine the lower and upper limits for the % GLs present in the mLNs of cats with normal intestine, IBD, small cell lymphoma (non-granular), and small cell granular lymphoma, in order to facilitate the diagnostic workup of cats with intestinal disease.

Hypothesis

Mesenteric lymph node aspirates of normal cats will comprise < 5% granular lymphocytes (GLs). More than 5% GLs will be associated with the presence of a clonal T-cell granular lymphocyte population and underlying intestinal granular T-cell lymphoma.

Methods

Wright-Giemsa staining

- Granular lymphocytes in Wright-Giemsa stained mLN aspirates were differentially counted at 100x objective in at least 10 high power microscopic fields, with a minimum of 500 cells counted.

Granzyme B immunostaining

- The accuracy of granular lymphocyte differential cell counts in mLN aspirates was confirmed using granzyme B immunocytochemical staining.

T cell molecular clonality PCR

- DNA was extracted from mLN aspirate slides, and T cell molecular clonality PCR was performed in triplicate using consensus TCRγ gene locus primers. This confirmed either lymphoma or normality / reactivity in each node.

Intestinal Biopsy Evaluation

- When available, accompanying intestinal biopsies were assessed morphologically and interrogated using granzyme B immunohistochemistry and T cell molecular clonality PCR to confirm the pathologic diagnosis.

Results

Results from each cytology and biopsy were correlated, and each case was sorted into one of five categories. The median % GLs counted in the mLNs of each category were as follows:

- Normal intestine & polyclonal node = **0.24%** (0.00-0.63%)
- IBD & polyclonal node = **0.29%** (0.00-1.11%)
- SCL non-granular & polyclonal node = **1.56%** (0.18-4.04%)
- SCL non-granular & clonal node = **1.15%** (0.15-6.29%)
- Clonal node w/ ↑ GLs, no biopsy = **6.68%** (2.78-57.51%)

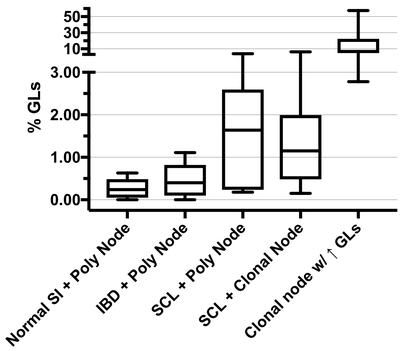
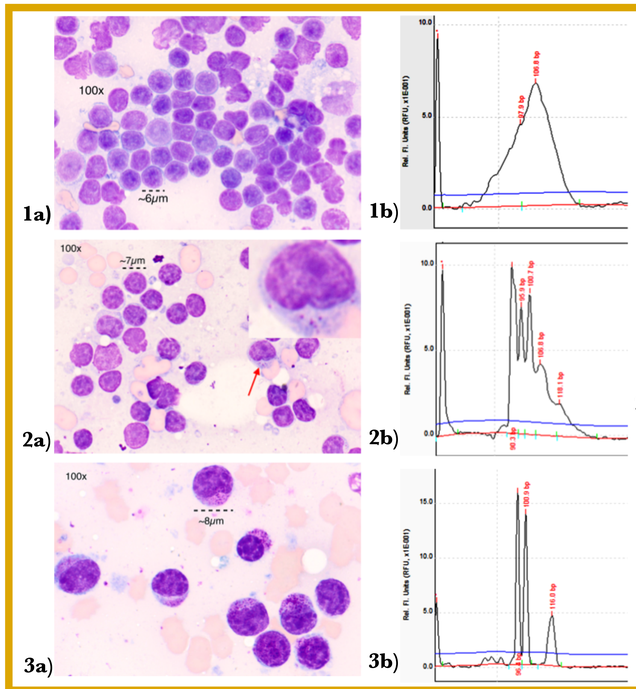


Figure 5: % granular lymphocytes present in mesenteric lymph nodes representative of various intestinal + nodal diagnostic categories.



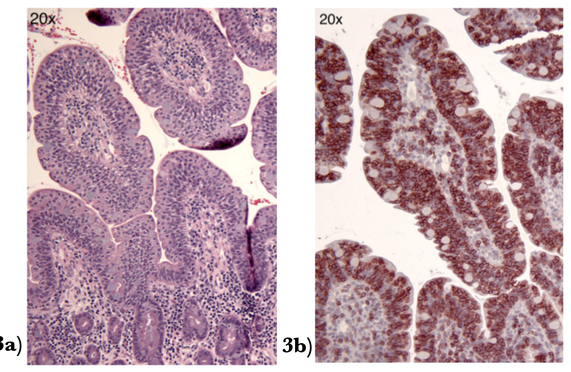


Figure 1: mLN aspirate with 0% granular lymphocytes, Wright-Giemsa staining (a); representative polyclonal PCR result (b).
Figure 2: mLN aspirate with 5% granular lymphocytes, Wright-Giemsa staining, (a); representative clonal PCR result with inflammatory background (b).
Figure 3: mLN aspirate with >25% granular lymphocytes, Wright-Giemsa staining (a); representative clonal PCR result (b).
Figure 4: intestinal biopsy diagnostic of epitheliotropic T cell lymphoma of granular lymphocyte type. H&E stain (a), CD3 immunostaining (b).

Discussion

Inflammatory bowel disease and intestinal lymphoma are common problems in cats, and they may present with similar clinical signs. Distinguishing IBD and intestinal lymphoma can be difficult. Clinicians frequently aspirate mesenteric lymph nodes when there is clinical concern for underlying intestinal lymphoma, as these lymph nodes are the first sites of distal metastasis from the intestine. This study confirmed that normal mesenteric nodes and nodes draining inflamed intestine contain a very low % of granular lymphocytes (0.00%-1.11%). Nodes that have involvement with either small cell lymphoma or small cell lymphoma of granular lymphocyte type have an increased % of granular lymphocytes (0.15%-57.51%). Intestinal lymphoma does not necessarily spread to the draining mesenteric nodes. Thus, lack of cytologic abnormalities in node aspirates does not exclude the possibility of underlying intestinal lymphoma. ~30% of the confirmed cases of intestinal small cell lymphoma have polyclonal mesenteric nodes. While these nodes are not neoplastic, they still contain more GLs (0.18%-4.04%) than normal nodes or nodes draining IBD. Therefore, when >1.50% of small granular lymphocytes are present in a mesenteric node aspirate, there should be a strong concern for underlying intestinal lymphoma, and further diagnostics are warranted. Such procedures could include molecular clonality PCR and/or intestinal biopsy. Because a full diagnostic workup is time-consuming, expensive, and invasive, the cytologic guidelines from this study will help clinicians decide whether further diagnostics are indicated. Additionally, some owners decline a full workup due to the cost, so cytologic guidelines that suggest a certain likelihood of underlying intestinal lymphoma are also useful in cases where further diagnostics are declined.

Future Directions

Accruing cases with mesenteric lymph node aspirates and underlying confirmed intestinal granular small cell lymphoma on biopsy would be useful. This would help to further differentiate the finding of increased GLs in mesenteric node aspirates. While many node aspirates in the "clonal node w/ increased GLs" contained a very high % of granular lymphocytes, there were no accompanying biopsies available for histopathologic confirmation of associated small cell lymphoma type.

The presence of such low numbers of GLs in normal mesenteric nodes and those associated with IBD is interesting. This may be because GLs in this region are most often tissue resident memory T-cells that don't traffic to local nodes. Interrogation of mLN aspirates with markers of tissue resident memory T cells to see how many are present in normal nodes would be useful.

References

1. Carreras JK, Goldschmidt M, Lamb M, McLear RC, Drobatz KJ and Sorenmo KU: Feline epitheliotropic intestinal malignant lymphoma: 10 cases (1997-2000). J Vet Intern Med 17: 326-331, 2003.
2. Louwerens M, London CA, Pedersen NC and Lyons LA: Feline lymphoma in the post-feline leukemia virus era. J Vet Intern Med 19: 329-335, 2005.
3. Moore PF, Rodriguez-Bertos A and Kass PH: Feline Gastrointestinal Lymphoma: Mucosal Architecture, Immunophenotype, and Molecular Clonality. Vet Pathol. 49(4):656-68, 2012.
4. Moore PF, Woo J, Vernau W, Kosten S and Graham P. Characterization of feline T cell receptor gamma (TCRG) variable region genes for the molecular diagnosis of feline intestinal T cell lymphoma. Vet Immunol Immunopathol. 106: 167–178, 2005.
5. Roccabianca P, Vernau W, Caniatti M and Moore PF: Feline large granular lymphocyte (LGL) lymphoma with secondary leukemia: primary intestinal origin with predominance of a CD3+/CD8(alpha)(alpha) phenotype. Vet Pathol 43: 15-28, 2006.