Feline Small Cell Lymphosarcoma Versus Inflammatory Bowel Disease: Diagnostic Challenges

Suliman Al-Ghazlat, DVM, DACVIM
Christian Eriksson de Rezende, DVM, MS
Jean Ferreri, DVM, DACVIM
BluePearl Veterinary Partners
New York, New York

Abstract: Inflammatory bowel disease (IBD) and small cell lymphosarcoma (SCLSA) are common causes of chronic gastrointestinal (GI) tract disease in cats. The history, clinical signs, and results of blood work and imaging for these conditions are nonspecific and often overlap. After a thorough diagnostic workup and treatment trials to rule out other conditions, a definitive diagnosis requires histopathologic evaluation of GI tract biopsy specimens. Full-thickness tissue samples appear to be superior to endoscopic biopsy samples in providing an accurate diagnosis. Adding advanced diagnostics such as polymerase chain reaction and immunohistochemistry to traditional histopathology may improve the diagnostic utility of small samples such as the ones obtained via endoscopy. Treatment of and prognosis for IBD and SCLSA are discussed in a companion article.

Inflammatory bowel disease (IBD) refers to a group of idiopathic, chronic gastrointestinal (GI) tract disorders characterized by persistent or recurrent GI signs and inflammation of the GI tract.1,2 The etiology of IBD is multifactorial and is, at least in part, mediated via inappropriate and uncontrolled inflammation of gut-associated lymphoid tissue against harmless environmental antigens such as those present in food and commensal bacteria.1–4 IBD was first reported in small animals around 25 years ago; today, it is the most common diagnosis in cats and dogs with chronic GI tract disease.1,2 The lack of clinical, diagnostic, histopathologic, and therapeutic standards has resulted in great challenges in the diagnosis and treatment of companion animal GI tract disorders and raised concerns regarding diagnosing IBD. The World Small Animal Veterinary Association (WSAVA) International GI Standardization Group and the American College of Veterinary Internal Medicine (ACVIM) have published multiple statements to provide guidelines and standards for performance and interpretation of various diagnostic tests in dogs and cats with GI diseases, including treatment trials, patient response, and outcome.2,5 These statements stress that IBD should not be diagnosed based on histopathology alone and that obtaining biopsy samples from the GI tract is only one part of a comprehensive workup (Box 1 and Box 2).

Lymphosarcoma (LSA) is the most common type of hematopoietic neoplasia in cats.6 Risk factors for feline LSA include FeLV or FIV infection, chronic inflammation, and exposure to cigarette smoke.6–9 However, most cats with GI tract LSA are negative for FeLV/FIV infection.6 Helicobacter infection may also play a role in the pathogenesis of the disease.10 LSA can occur in multiple anatomic locations, but the GI tract is the most common in cats.6 Over time, there has been an apparent increase in localization of LSA to the GI tract in cats.6–11 Clinically and histologically, LSA is divided into lymphocytic, low-grade, small cell LSA (SCLSA) or lymphoblastic, high-grade, large cell LSA (LCLSA).12,13 A separate, rare histologic type of GI tract disease (SCLSA) is associated with a poor outcome and is not responsive to conventional treatment.12

For more information, please see the companion article, “Feline Small Cell Lymphosarcoma Versus Inflammatory Bowel Disease: Treatment and Prognosis.”

Box 1. Criteria for the Clinical Diagnosis of Inflammatory Bowel Disease in Dogs and Cats

- Chronic persistent or recurrent GI tract signs for ≥2 weeks
- Inability to document other causes of GI signs or and inflammation
- Inadequate response to dietary, antimicrobial, and anthelmintic therapies alone
- Histopathologic evidence of mucosal inflammation
- Clinical response to antiinflammatory or immunosuppressive agents

These criteria do not differentiate inflammatory bowel disease from lymphosarcoma.
Box 2. Suggested Workup for Cats With GI Tract Disease Before Biopsy

- Perform a complete blood count, biochemistry profile, urinalysis, and thyroid evaluation to look for underlying or concurrent diseases.
- Perform fecal testing for GI parasites.
- Perform abdominal ultrasonography to evaluate the extraintestinal organs in addition to GI tract wall thickness, layering, and motility.
- Measure vitamin B₁₂ level to assess disease severity and localization and the need for supplementation.
- Perform treatment trials for food intolerance and GI parasites when appropriate in stable patients.
- Measure fPLI to test for pancreatitis in cats with vomiting and/or decreased appetite.
- Measure fTLI to test for exocrine pancreatic insufficiency in cats with appropriate in stable patients.
- Perform thoracic radiography to look for evidence of metastatic disease or concurrent cardiac or pulmonary diseases.
- Measure vitamin B₁₂.
- Perform abdominal ultrasonography to evaluate the extraintestinal organs.
- Perform fecal testing for GI parasites.
- Thyroid evaluation to look for underlying or concurrent diseases.
- Perform abdominal radiography to look for evidence of metastatic disease or concurrent cardiac or pulmonary diseases.
- Measure vitamin B₁₂ level to assess disease severity and localization and the need for supplementation.
- Perform treatment trials for food intolerance and GI parasites when appropriate in stable patients.
- Measure fPLI to test for pancreatitis in cats with vomiting and/or decreased appetite.
- Measure fTLI to test for exocrine pancreatic insufficiency in cats with appropriate in stable patients.
- Perform thoracic radiography to look for evidence of metastatic disease or concurrent cardiac or pulmonary diseases.

IBD and LSA are often the remaining differentials for a feline patient with chronic GI tract signs after a thorough, noninvasive diagnostic workup including therapeutic trials (Box 2). The clinical history and findings from the physical examination; hematology, biochemistry, and imaging results; and even histopathologic changes of IBD and GI tract SCLSA often overlap.

Signalment
There is no clear sex, age, or breed predisposition for feline IBD or LSA. However, in some studies, males appear to be predisposed to GI tract LSA. Cats with LSA tend to be older, with a median age of 9 to 13 years; however, GI tract LSA has been reported in cats 1 to 20 years of age.

Clinical Signs
The common clinical signs of LSA and IBD are nonspecific and include any combination of weight loss, variation in appetite, vomiting, diarrhea, and lethargy. Abnormalities in both conditions may include thin body condition with muscle wasting, evidence of dehydration, thickened intestinal loops, and mesenteric lymphadenopathy. In cats with LCLSA, abdominal masses may be palpated.

Clinical Pathology
Laboratory findings are often nonspecific; mild nonregenerative anemia, neutrophilia, and mild hypoalbuminemia have been frequently reported in cats with LSA. Hypoalbuminemia is rare in cats with IBD. Hypocobalaminemia is a frequent finding in both conditions and is an important therapeutic target.

Imaging
Abdominal radiography is rarely useful in the diagnosis. Abdominal ultrasonography findings can be normal in some cases of IBD or LSA. Common ultrasonographic findings in both conditions include generalized or regional gastric or intestinal wall thickening, mesenteric lymphadenopathy, and decreased motility. Findings that suggest a neoplastic process and that should increase the indications for biopsy include loss of normal wall layering, disproportionately thick muscularis propria, focal intestinal mass effects, and ascites.

Biopsy and Histopathology
A thorough diagnostic workup to rule out other causes is crucial. However, to confirm the diagnosis, histopathologic evaluation of appropriately collected GI tissue samples is required. Problems in making the diagnosis may be encountered because of overlap in histologic features between IBD and LSA (predominantly SCLSA), differences of opinion among pathologists, inadequacy of tissue specimens, and the fact that if the pathologic process is segmental, sampling the wrong segment of the GI tract can result in misdiagnosis. In addition, LSA and inflammatory infiltrates can coexist. The potential for progression of IBD to LSA further complicates the diagnosis. Mucosa-associated lymphoid tissues (MALT) are normally populated primarily by T lymphocytes, and in both IBD and SCLSA, the T-cell population expands beyond the MALT. However, in contrast to IBD, neoplastic cells frequently infiltrate beyond the mucosa, destroying normal tissue architecture. Therefore, biopsy specimens of intestinal LSA in which neoplastic cells do not extend beyond the mucosa and endoscopic biopsy specimens, in which histologic evaluation is often limited to the mucosa, are especially difficult for pathologists to interpret.

For clinicians, the most challenging question associated with GI tract biopsy is how to obtain tissue samples of adequate depth and at the correct anatomic location. Whether to obtain full-thickness biopsy (FTB) or endoscopic biopsy (EB) samples has been a subject of discussion over the past decade. Benefits of EB include the ability (1) to see mucosal changes and lesions, (e.g., ulceration, erosion, lymphangiectasia), enabling directed biopsy of these sites; (2) to collect multiple tissue samples (e.g., 10 or more) from each site, as some diseases may have a multifocal distribution, even within the same section of the intestine; and (3) to begin antiinflammatory or chemotherapeutic agents without delay after the procedure. On the other hand, EB is limited to...
the proximal GI tract (stomach and duodenum) and colon and distal ileum.

Surgery or laparoscopy permits the collection of transmural samples of any segment of the GI tract in addition to samples from other organs (lymph nodes, liver, and pancreas). In rare cases of intestinal perforation or obstruction, surgery can also have a therapeutic role.

**Full-Thickness Versus Endoscopic Biopsy Samples**

In 2006, Evans et al\(^2\) concluded that EB samples are not adequate for differentiating IBD from LSA in the small intestine. In a prospective study of 22 cats with IBD or GI tract LSA, EB specimens of the stomach and duodenum were obtained immediately before laparotomy or laparoscopic surgery, during which FTB specimens were obtained from the stomach, duodenum, jejunum, and the ileum. LSA was diagnosed in 10 cats based on analysis of the FTB specimens. LSA was detected in the jejunum and ileum in all 10 of these cats and in the duodenum in nine cats. However, based on analysis of the EB samples, of the nine cats with LSA in the duodenum, one had a diagnosis of LSA, three had findings suggestive of LSA, and five had a diagnosis of IBD. This is the most commonly cited study to show the superiority of FTB over EB. It highlights some of the serious limitations of endoscopy, as of the five cats with LSA diagnosed as IBD on duodenal EB, two underwent only partial duodenal assessment and in three, duodenal biopsy had been performed blindly. Overall, in eight out of 22 cats, the pylorus could not be passed with the endoscope. and three duodenal samples were obtained blindly. Despite this study’s findings, the 2010 ACVIM Consensus Statement recommended that where biopsy of the GI tract is indicated, EB is the preferred choice.\(^2\)

Kuipel et al published a retrospective study on jejunal and ileal tissue samples (50 FTB, 13 EB) collected from a total of 63 cats.\(^2\) In 39 cases, the lymphocytic infiltration extended into the submucosa; 95% (37 of 39) of these cases were diagnosed as LSA. Lymphocyte infiltration was seen in 28 of 50 tunica muscularis samples; 27 of these cases were diagnosed as LSA. Of 49 samples that included serosa, 22 had serosal infiltration, and all 22 of these cases were diagnosed as LSA.

Because infiltration of lymphocytes deeper than the mucosa is a major criterion for LSA diagnosis, FTB specimens appear to be superior to EB specimens for differentiating LSA from IBD. On the other hand, in many cats diagnosed with LSA in the Kuipel et al study, the infiltrate was limited to the mucosa. For example, intramuscular infiltrates were present in only 20 of 34 cases of LSA that were diagnosed from FTB specimens.\(^2\) In other words, although tunica muscularis infiltration has excellent specificity (96%) for LSA, it only occurred in 59% of cats with LSA. An earlier study by Kleinschmidt et al on 43 cats with inflammatory and noninflammatory diseases of the GI tract also showed a diagnostic advantage for FTB samples.\(^2\) This study also showed an advantage for sampling all the segments of the GI tract, the liver, and the mesenteric lymph nodes. Three out of 10 cats diagnosed with LSA in the Kleinschmidt study would have been misdiagnosed as IBD if samples were collected from only the stomach, duodenum, and colon.

**Duodenal Versus Ileal Endoscopic Biopsy Samples**

Recently, an excellent retrospective study strongly questioned the accuracy of obtaining endoscopic samples from the duodenum alone.\(^2\) In the study, a diagnosis of LSA was made in 18 of 70 cats based on duodenal and/or ileal EB samples. Eight cats were diagnosed with only ileal LSA, seven were diagnosed with only duodenal LSA, and three were diagnosed with LSA in the ileum and the duodenum. In other words, a standard gastroduodenoscopy in this population would have resulted in misdiagnosing 44% of cats. The study showed a clear diagnostic advantage to obtaining both duodenal and ileal tissues samples when endoscopy of the GI tract is performed.

**Immunohistochemistry and Polymerase Chain Reaction**

As discussed above, differentiation of GI tract SCLSA from IBD based on histopathology using only hematoxylin-eosin (HE) staining is problematic. Detection of a clonal population of cells (cells from a single origin) in a lymphoproliferative lesion is an important criterion for the diagnosis of neoplasia.\(^28,31\) Polymerase chain reaction (PCR) can be used to detect clonality in lymphocytes. PARR (polymerase chain reaction for antigen receptor rearrangement) tests for specific mutations in the lymphoid genome that are found in many cases of lymphoid neoplasia. This test is used to help make a definitive diagnosis of lymphoid neoplasia if results of other tests are equivocal.

Immunohistochemistry (IHC) is used clinically to immunophenotype cells with fluorescent-labeled antibodies raised against specific cell surface proteins to assess the phenotypic uniformity of a mucosal infiltrate.\(^26–28\) The pattern of positive and negative interactions with the various antibodies yields a diagnosis of cell lineage and, if applicable, lymphoid immunophenotype. Besides its value in reaching a diagnosis, immunophenotyping might be of prognostic value in cases of feline GI tract LSA.\(^22\) Both IHC and PCR can be performed on formalin-fixed tissue.

In 2005, a study was published in which samples from 32 cats with a histopathologic diagnosis of GI

**Key Points**

- IBD cannot be diagnosed based on histopathology alone. A thorough diagnostic workup and treatment trials are needed to rule out other causes for GI tract signs.
- The overlap in histologic features, differences of opinion among pathologists, inadequacy of tissue specimens, regional nature of the pathologic process and the fact that LSA and IBD can coexist make the differentiation between the two conditions very challenging.
- Clinicians should attempt to collect duodenal and ileal tissue samples when EB is performed.
- IHC and PCR are promising adjunctive diagnostics and will likely improve the ability to differentiate IBD from LSA.
tract LSA or multicentric LSA affecting the GI tract were reevaluated using histopathology and IHC. This reevaluation resulted in five cases being redefined as probable inflammatory enteropathy rather than LSA. This study suggested that IHC is a useful adjunct to histopathology and may help in distinguishing LSA from severe GI tract inflammation.

In another study published in 2005, PCR was used to determine molecular clonality in samples from cats with diagnosed intestinal IBD (nine cats), or transmural and mucosal T-cell LSA (28 cats). Clonal rearrangement was detected in 22 of 28 cases of intestinal T-cell LSA, and oligoclonality was detected in three cases of intestinal T-cell LSA. In contrast, polyclonal rearrangement was detected in normal intestinal tissues (three cats) and in all nine cats with IBD. If the clonal and oligoclonal results are combined, the sensitivity of clonality detection was 89%. Failure to detect clonal T-cell populations occurred in three cats (11%) with T-cell LSA. The study showed that the sensitivity of the PCR assay makes it useful for the detection of clonal rearrangements in small amounts of tissue, such as EB samples.

Combining Histopathology, Immunohistochemistry, and Polymerase Chain Reaction Testing

The main goal of Kiupel’s study was to evaluate the impact of adding IHC and PCR results for lymphocyte clonality to the routine diagnosis of LSA or inflammation based on microscopic examination of HE stained sections alone. All of the patients were diagnosed with either IBD (19 cases, 30%) or intestinal LSA (44 cases, 70%), based on routine histopathologic examination alone. Ten of the 19 cases (53%) that originally had been diagnosed as IBD by histopathologic examination alone were reclassified as LSA when IHC and PCR results were evaluated in conjunction with the HE findings. Three of the 47 cases (6%) that originally had been diagnosed as LSA by histopathologic examination alone were reclassified as IBD when IHC and PCR results were added. The study demonstrates that combining the histopathologic evaluation with IHC of the lymphocytic infiltration and analysis of clonality by PCR results in a more accurate differentiation of neoplastic from inflammatory lymphoid cells.

Summary

Differentiating low-grade SCLSA from IBD in cats is challenging. Based on the current literature, owners should be educated about the likely superiority of FTB over EB in obtaining an accurate diagnosis. In our experience, after this discussion, many owners still choose to proceed with EB for reasons related to perceived invasiveness, the critical nature of some patients, and cost. When endoscopy is performed, obtaining duodenal and ileal samples should be attempted. Systematic assessment of samples using routine histopathology, IHC, and PCR may help decrease the likelihood of misdiagnosis, especially with endoscopic samples.

References


1. Which of the following is not a criterion for the diagnosis of IBD?
   a. histopathologic evidence of mucosal inflammation
   b. inability to document other causes of gastrointestinal signs and/or inflammation
   c. chronic persistent or recurrent GI tract signs (for ≥2 weeks)
   d. a complete clinical response to a well-designed dietary trial

2. Which statement is correct regarding LSA in cats?
   a. Small-cell LSA (SCLSA) is an acute disease with short clinical history and rapid deterioration.
   b. Most cats with GI tract LSA are positive for FeLV.
   c. The most common anatomic location for feline LSA is the GI tract.
   d. It is often difficult to differentiate SCLSA from large-cell LSA based on histopathology.

3. Which of the following statements is correct regarding the diagnostic workup for a cat suspected of having SCLSA or IBD?
   a. It is usually easy to differentiate between the two conditions based on the history and the physical examination.
   b. Abdominal ultrasonography can usually differentiate SCLSA from IBD.
   c. A normal abdominal ultrasound can rule out SCLSA.
   d. Increased thickness of the muscularis layer on ultrasonography is suggestive of SCLSA.

4. Which of the following statements is false with regard to surgical exploration?
   a. It allows full-thickness biopsy of the GI tract.
   b. It is the method of choice to diagnose gastric ulcers.
   c. It allows biopsy of organs outside the GI tract.
   d. Samples obtained during this procedure may be superior to EB for differentiating IBD from SCLSA.

5. When endoscopy is performed in a cat to confirm the diagnosis of IBD versus SCLSA, the clinician should attempt to collect samples from which locations?
   a. stomach only
   b. stomach and jejunum
   c. jejunum and duodenum
   d. duodenum and ileum

6. Which statement is false with regard to the diagnosis of GI tract endoscopy in veterinary medicine?
   a. It allows the collection of multiple mucosal samples of all GI tract segments.
   b. It is less invasive than laparoscopy and surgery.
   c. It allows the clinician to directly diagnose lesions such as ulcers and lymphangiectasia.
   d. It allows initiation of antiinflammatory or chemotherapeutic therapy directly after the procedure.

7. Histopathologic differentiation between IBD and SCLSA is challenging because
   a. the histologic features of IBD and SCLSA overlap.
   b. pathologists’ interpretations can differ.
   c. SCLSA and inflammatory infiltrates can coexist.
   d. all of the above

8. Which statement is false with regard to polymerase chain reaction (PCR) testing?
   a. It can be used to detect clonality in lymphocytes.
   b. The sensitivity of PCR makes it useful for the detection of clonal rearrangements in small amounts of tissue.
   c. If PCR fails to detect clonality, LSA can be ruled out.
   d. It is a promising test to help differentiate IBD from LSA.

9. Immunohistochemistry (IHC) may be useful for
   a. determining the phenotypic uniformity of lymphocytes.
   b. differentiating lymphocytic inflammation from neoplasia.
   c. obtaining more information regarding the prognosis of LSA.
   d. all of the above

10. Which statement regarding diagnosis of IBD versus LSA is most accurate?
    a. Histopathology of hematoxylin-eosin-stained samples always differentiates IBD from LSA.
    b. Studies have proved that full-thickness and endoscopic biopsy samples are equivalent for diagnostic testing using histopathology.
    c. PCR and IHC results may change initial diagnoses based on histopathology alone.
    d. Biopsy results alone are adequate for diagnosing IBD or LSA.