Utility of Endoscopic Biopsies of the Duodenum and Ileum for Diagnosis of Inflammatory Bowel Disease and Small Cell Lymphoma in Cats

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Background: Endoscopic duodenal biopsies are relatively convenient, minimally invasive tests for infiltrative intestinal disorders of cats. Ileal endoscopic biopsies might not be performed because of technical difficulty and effort required to prepare the colon. It is not known whether or not histopathology of feline duodenal and ileal biopsies for detection of inflammatory bowel disease (IBD) and small cell lymphoma (SC-LSA) provides comparable results.

Objectives: To evaluate the agreement between endoscopic biopsies of duodenum and ileum in cats with IBD and SC-LSA.

Animals: Seventy client-owned cats with gastrointestinal disease and adequate duodenal and ileal tissue biopsies obtained endoscopically.

Methods: Retrospective study: Search of medical records of cats with enteropathy and endoscopy. Samples were blinded and re-evaluated by single pathologist (JM) for quality, number of biopsies, and diagnosis according to WSAVA standards. Agreement of IBD and SC-LSA diagnoses among biopsy sites assessed using Cohen’s Kappa.

Results: Eighteen of 70 cats (26%) were diagnosed with SC-LSA in duodenum, ileum, or both. Of these 18 cats, 7 (39%) were diagnosed with only duodenal SC-LSA, 8 (44%) were diagnosed with only ileal SC-LSA, and 3 (17%) had SC-LSA in both duodenum and ileum. There was poor agreement on diagnosis between duodenal and ileal biopsies (kappa = 0.23).

Conclusions and clinical importance: Although review by a single pathologist remains a limitation of this study, results suggest that there is a population of cats in which diagnosis of SC-LSA can be found only by evaluation of ileal biopsies. Clinicians should consider performing both upper and lower GI endoscopic biopsies in cats with infiltrative small bowel disease.

Key words: Gastroenterology; Histopathology; Small intestine.

Infiltrative small intestinal disorders of cats are commonly encountered in middle-aged or senior cats. Although true prevalence is unknown, common causes include idiopathic inflammatory bowel disease (IBD) or small cell lymphoma (SC-LSA).1,2 Other possible causes of inflammatory cell infiltrates include dysbiosis, chronic infection (histoplasmosis, toxoplasmosis, parasites), chronic food sensitivity, or food intolerance.3 These disorders usually result in diarrhea, vomiting, and weight loss that range from mild to severe. Definitive diagnosis of inflammatory bowel disease (IBD) and SC-LSA requires small intestinal biopsy with histopathologic analysis as well as eliminating other known causes of intestinal inflammation.1,4

Endoscopic biopsies are a convenient, minimally invasive test for infiltrative intestinal disorders of cats. Gastroduodenoscopy allows for collection of gastric, duodenal, and possibly jejunal intestinal samples. Ileal samples can also be obtained endoscopically in a retrograde manner, but require preparation of the colon using one or more of lavage solutions, cathartics, and enemas. Commonly, only the duodenum and jejunum are examined because of custom, cost, and the risks and time required to prepare the colon.

There is poor agreement between duodenal and ileal histopathology in dogs with enteropathy.5 Information regarding the importance of histopathology obtained from different areas of the small intestine of cats is lacking. Therefore, it is unknown if obtaining ileal biopsies endoscopically might provide additional information for the diagnosis of infiltrative intestinal disease. The purpose of this study was to evaluate the agreement between endoscopic biopsies of the duodenum and ileum in cats with infiltrative intestinal disease.

Abbreviations:

IBD inflammatory bowel disease
SC-LSA small cell lymphoma
WSAVA World Small Animal Veterinary Association
Methods

Medical records from 1994 to 2007 at the Texas A&M College of Veterinary Medicine were retrospectively reviewed for cats that had undergone endoscopic intestinal biopsies. Of these, 93 cases were available, and 74 cats met the inclusion criteria of having presented for gastrointestinal disturbances, ultimately undergoing endoscopic examination with biopsy samples obtained from different areas of both the duodenum and ileum, and having slides from both duodenum and ileum available for reviewer evaluation. Biopsies of the ileum were either obtained by intubation and direct visualization of biopsy collection or by passing biopsy forceps blindly into the ileum for sample collection. Because of the retrospective nature of this article, method of ileal biopsy collection was not known for all cases. Duodenal biopsies were obtained by intubation and direct visualization. Exact intraluminal location of duodenal and ileal biopsies were not recorded. All slides evaluated contained either duodenum or ileum samples, but not both on the same slide. These slides were randomized through use of a table of randomized numbers and reviewed by a single pathologist (JM). Duodenal and ileal biopsies from each case were analyzed without knowledge of results from the other location. No information regarding history, clinical signs or endoscopic findings was made available to the pathologist. Samples were evaluated for quality (inadequate, marginal, adequate), number of biopsies available, and diagnosis according to World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group standards. Cats were excluded if only inadequate samples were available for analysis in either the duodenum or ileum.

All samples were evaluated for presence of 5 morphologic features—villous stunting, epithelial injury, crypt distention, lacteal dilatation, and fibrosis. Samples were also evaluated for presence of inflammatory cells—intraepithelial lymphocytes, lamina propria lymphocytes, lamina propria eosinophils, lamina propria neutrophils, or other inflammatory cells. Each of these 10 features was categorized as normal, mild, moderate, or severe according to WSAVA Gastrointestinal Standardization Group standards. A final diagnosis was determined for each sample from 8 different categories of diagnosis. These included no abnormalities detected (NAD), lymphoplasmacytic inflammatory, eosinophilic inflammatory, neutrophilic inflammatory, lymphangiectasia, large cell lymphoma, small cell lymphoma, mucosal atrophy/fibrosis (non-inflammatory) or other. The pathologist was also given the option to write in additional comments. Inflammatory infiltrates in the intestine were categorized using WSAVA Gastrointestinal Standardization Group standards, and a histologic diagnosis of IBD was assigned in the presence of these inflammatory changes without evidence of other disease. Small cell lymphoma was differentiated from inflammatory bowel disease by the presence of a monomorphic population of small lymphocytes in the absence of plasma cells, causing morphologic thickening, blunting and sometimes fusion of villi and distention of the lamina propria with or without infiltration into the submucosa, and marked infiltration of lymphocytes into the epithelium. If the degree of inflammation was severe enough to raise suspicion for possible SC-LSA, this was documented. Cats with a diagnosis of severe intestinal inflammation with possible SC-LSA were grouped as possible SC-LSA. Cats with SC-LSA in 1 region of the intestine but a different diagnosis in the other region were classified as having SC-LSA. Cats with IBD in 1 region of the intestine but normal tissue in the other regions were classified as having IBD. Cats with possible SC-LSA in 1 region of the intestine but normal or IBD diagnosis in the other regions were classified as having possible SC-LSA.

Data were stored in a commercially available spreadsheet program (MS Excel 2007). A commercially available statistical software program was used to calculate descriptive statistics and for analytical comparisons. The fractions of cats with a diagnosis of LSA, inflammation, or both, overall and by intestinal location, were reported as proportions and exact 95% confidence intervals (CIs). Descriptive statistics for continuous variables, including age at diagnosis and number of biopsy samples, were presented as means and standard deviations or minimum, maximum, and 25th, 50th, 75th percentiles as appropriate. Dichotomous variables were presented as counts and percentages. Comparison of biopsy quality was done using McNemar’s test (P < .05 was considered significant). Agreement between diagnoses of LSA, inflammation, and normal by intestinal location was assessed by calculating Cohen’s kappa coefficient. Kappa values between 0 and 0.40 were characterized as poor, 0.41–0.75 as fair to good, and greater than 0.75 as excellent agreement.

Results

Four cases were excluded because of inadequate samples in the duodenum, ileum, or both, leaving 70 cases available for analysis. The mean age (±SD) of the 70 cats by sex was 10.4 (±4.3) years and 10.3 (±3.7) years for females (n = 29) and males (n = 41), respectively. The average age (±SD) was 11.3 (3.6) years for SC-LSA, 10.5 (4.2) years for IBD, and 8.1 (3.0) years for normal cats. Forty cats were neutered males, 28 spayed females, 1 intact male, and 1 intact female. The majority of cats were Domestic Shorthair (46) or Longhair (13) breeds. Other represented breeds included Persian (3), Balinese (2), Maine Coon, Ocicat, Tonkinese, Himalayan, Bombay, and Angora cats (1 each).

In all, 987 duodenal tissue pieces were analyzed on available slides, with a median of 12 and range of 3–39 per case. The ileal tissue pieces that were analyzed on available slides with a median of 6 and range of 1–27 per case numbered 522. For duodenal biopsies of included cases, 68 of 70 cases (97%) had biopsies of adequate quality and 2 of 70 (3%) were marginal. For ileal biopsies of included cases, 59 of 70 cases (84%) had biopsies of adequate quality whereas 11 of 70 (16%) were marginal. Biopsies classified as marginal were significantly more likely (P = .013) to occur in samples taken from the ileum.

Of the 70 cases ultimately included, 9 (13%) had normal biopsy results in both duodenum and ileum. Thirty-three of the 70 (47%) had only inflammation without evidence of SC-LSA or possible SC-LSA in either location. Nine of these 33 had only duodenal inflammation, 5 of 33 had only ileal inflammation, and 19 of 33 had both duodenal and ileal inflammation. Ten of the 70 (14%) were diagnosed with possible SC-LSA in the duodenum, ileum, or both. Three of these 10 had possible duodenal SC-LSA with normal or only mild IBD in the ileum. Eighteen of 70 (26%) were diagnosed with SC-LSA in the duodenum, ileum, or both. The overall agreement on all diagnosis between duodenal and ileal biopsies (Table 1) including normal, mild-severe inflammation, possible SC-LSA, and SC-LSA was poor (kappa = 0.23; P < .001).

Of the 18 cats with SC-LSA, 7 (39%) were diagnosed with only duodenal SC-LSA, 8 (44%) were
diagnosed with only ileal SC-LSA, and 3 (17%) had SC-LSA in both duodenum and ileum. There was also a poor agreement (kappa = 0.16; \( P = .09 \)) of SC-LSA diagnoses between duodenal and ileal biopsies (Table 2). Agreement improved slightly to fair to good (kappa = 0.41; \( P < .001 \)) when cats with normal or IBD, possible SC-LSA, and SC-LSA diagnoses in duodenal and ileal biopsies were evaluated (Table 3).

Of 7 cats diagnosed with only duodenal SC-LSA, 5 had severe inflammation in ileal biopsies, in which the pathologist had concerns that the inflammation was possibly consistent with early SC-LSA. Two of these 7 had ileal biopsies with only mild to moderate LP inflammation (1 of each), and were not consistent with SC-LSA. In 8 cats with only ileal SC-LSA, 3 had severe inflammation in duodenal biopsies, in which the pathologist had concerns that the inflammation was possibly consistent with early SC-LSA. Five of these 8 had duodenal biopsies without evidence of SC-LSA [normal (1), mild inflammation (1), moderate inflammation (1), and severe inflammation without suspicion for lymphoma (2)].

Discussion

Cats within this study population frequently had different endoscopic diagnoses depending on location of biopsies. Agreement between duodenal and ileal diagnoses of normal, inflammation (mild, moderate, severe), and SC-LSA was poor based on kappa analysis. Although agreement improved slightly when cats with possible SC-LSA were evaluated as an independent group from IBD, this study documents that histopathologic results from different areas of the intestine in cats with infiltrative intestinal diseases like IBD and SC-LSA are often inconsistent. This inconsistency can lead to misdiagnosis if only 1 area of the small intestine is biopsied.

There was also a poor agreement between SC-LSA diagnoses obtained in the duodenum and ileum. Most importantly, some cats were diagnosed with SC-LSA in the duodenum or ileum without any suggestion of SC-LSA in the other areas of the intestine. In 5 cats with ileal SC-LSA, duodenal diagnoses ranged from normal intestine to severe inflammation, but had no evidence or suspicion of SC-LSA. If a clinician had only biopsied the duodenum in these cases, a diagnosis of SC-LSA would have been missed. Likewise, 2 cases with duodenal SC-LSA had only mild and moderate IBD found in the ileum. Therefore, 7 of 18 cats with SC-LSA (38.9%) would have been misdiagnosed as not having SC-LSA if biopsies had only been obtained from 1 area of the small intestine.
Previous studies have questioned the adequacy of endoscopic biopsies when trying to diagnose feline inflammatory bowel disease or lymphoma. However, in one of these studies the duodenum was not entered endoscopically and few biopsies were obtained. In addition, this study was published before the current WSAVA guidelines, and sample quality was not described. Therefore, it is unknown if poor quality or low number of samples may have contributed to study findings. Full thickness biopsies allow for better determination of the extent of lymphocyte infiltration, which may extend beyond the mucosa and submucosa in some cases of SC-LSA, and should remain more superficial in IBD. However, obtaining full thickness biopsies requires laparoscopy or laparotomy, procedures that are more invasive than endoscopy. Laparotomy requires an extended recovery time, and both procedures are generally associated with higher client costs, some degree of perioperative pain and risk of intestinal dehiscence. Results of this study suggest that diagnostic inconsistency attributable to sampling only 1 intestinal location endoscopically may be responsible for some of the reported inadequacy of endoscopic biopsies differentiating between IBD and SC-LSA. It is likely that evaluating both duodenal and ileal endoscopic biopsies would improve the diagnostic sensitivity of endoscopic biopsies in the diagnosis of IBD and SC-LSA. Because ileal biopsies were found in this study to be very important in the diagnosis of SC-LSA, the next question will be how beneficial jejunal biopsies are in the diagnosis of SC-LSA. If jejunal samples are found to be an important addition when searching for SC-LSA, then the question will be whether the proximal jejunum (which may be accessible endoscopically in cats) is satisfactory or if mid-jejunal samples are necessary.

Ileal biopsies in this study were significantly more likely than duodenal biopsies to be classified as “marginal” in quality. This difference in biopsy quality is most likely related to the techniques used when collecting biopsies. Intubation of the duodenum with the endoscope is commonly achieved, and allows for direct visualization of the small intestine during the biopsy process. Intubation of the ileum is more challenging and may require the use of a smaller diameter endoscope or collection of ileal biopsies by blindly passing biopsy forceps through the ileocolic sphincter, which may result in excessive damage to the ileal mucosa. The ileal biopsies collected in this study were most commonly collected using this “blind” technique, which most likely accounted for the difference in quality. However, 3 or more tissue samples were available for analysis for all cases with only marginal ileal biopsies. Based on a previous study evaluating endoscopic biopsy quality and sensitivity, having at least 3 marginal tissue samples allows for a 90% confidence in diagnosis of mild to moderate duodenal infiltrates in cats.

Limitations of this study primarily include its retrospective nature. The biopsies were collected by different clinicians, which may have produced some variation in quality. However, quality and number of samples available for analysis were determined, and cases were excluded if appropriate diagnostic standards were not met. This study did not evaluate full thickness biopsies obtained via laparotomy or laparoscopy, which may be a preferred diagnostic tool for infiltrative intestinal disease and may have limited the ability of the pathologist to distinguish between IBD and SC-LSA. This study also did not evaluate jejunal biopsies, which may have provided additional information regarding agreement between biopsies from different locations in the intestinal tract. Further research into agreement in diagnoses between endoscopic and full thickness biopsies, as well as agreement from all areas of the gastrointestinal tract in cats with infiltrative intestinal diseases, is still needed.

Because there were instances in which a diagnosis of severe IBD could not clearly be distinguished from possible SC-LSA, we classified this group of patients as having “possible SC-LSA”. All the cats with possible SC-LSA had biopsies classified as “adequate”, so decreased sample quality was unlikely to contribute to this diagnosis. By excluding these cats from the SC-LSA group and leaving them within the possible SC-LSA group, we hoped to prevent bias in our study caused by inaccurate grouping of diagnoses. Recently it has been shown that combining histologic evaluation of feline intestinal biopsies with immunophenotyping and PCR to determine clonality of lymphocytes allows for more accurate differentiation between IBD and SC-LSA. Immunophenotyping and PCR were not consistently performed in these cases, and might have improved overall diagnostic accuracy.

A potentially important study limitation is that only 1 pathologist evaluated the slides in this study, and it has been previously documented that pathologists can have substantial differences in opinion in the diagnosis of intestinal disease of dogs and cats. However, that study looked at the effect of pathologist on diagnosis of normal, mild, moderate, and severe inflammation and neoplasia. It did not specifically evaluate this effect on diagnosis of small cell intestinal lymphoma in cats. The fact that the pathologic diagnoses in this report were based on WSAVA standards might have eliminated some of the risk of disagreement, even if a second pathologist had looked at these slides, it could still be argued that immunohistochemistry is necessary to definitively diagnose small cell lymphoma in intestines of cats. Thus, while having 2 pathologists would give some additional confidence, it would not totally resolve the problem. In addition, the point of this article is that lymphoma can be diagnosed in the ileum when it cannot be diagnosed in the duodenum. This study found 8 cats in which only the ileum showed evidence of lymphoma. Even if one were to assume that up to 50% of these diagnoses were erroneous, it would still leave approximately 20% of all cats with SC-LSA requiring an ileal biopsy for diagnosis.

Results of this study suggest that location of endoscopic biopsy collection is important when trying to diagnose feline intestinal inflammation or lymphoma.
Our results suggest that there is a population of cats in which diagnosis of SC-LSA may only be found by evaluating ileal biopsies. Clinicians should consider performing both upper and lower GI endoscopic biopsies in cats with suspected infiltrative small bowel disease.

Footnote

a Stata 11.0, StataCorp, College Station, TX

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References