Original Article

jfms Journal of Feline Medicine and Surgery

Fecal S100A12 concentrations in cats with chronic enteropathies

Journal of Feline Medicine and Surgery
1–11

DOI: 10.1177/1098612X231164273
journals.sagepub.com/home/jfm

This paper was handled and processed by the American Editorial Office (AAFP) for publication in *JFMS*

SAGE

Kailee A Zornow[1], Jennifer E Slovak[1], Jonathan A Lidbury[2], Jan S Suchodolski[2] and Joerg M Steiner[2]

Abstract

Objectives The aim of this study was to compare fecal S100A12 concentrations in cats diagnosed with chronic enteropathy (CE) with healthy control cats.

Methods This was a prospective, cross-sectional study. Forty-nine cats that had gastrointestinal signs for >3 weeks and a complete diagnostic work-up, including bloodwork, abdominal ultrasound and upper and/or lower gastrointestinal endoscopic biopsies, were enrolled into the CE group. Nineteen cats from the CE group were diagnosed with inflammatory bowel disease (IBD) or chronic inflammatory enteropathy (CIE) and 30 with alimentary lymphoma (LSA), based on histopathology results and additional testing with immunohistochemistry or molecular clonality testing with PCR if indicated. Nineteen apparently healthy control cats were included in the study. One fecal sample was collected from each cat and S100A12 concentrations were quantified by an analytically validated in-house ELISA.

Results Fecal S100A12 concentrations differed between cats with LSA (median 110 ng/g; interquartile range [IQR] 18-548) and control cats (median 4 ng/g; IQR 2-25 [$P < 0.001$]) and between cats with IBD (median 34 ng/g; IQR 15–973) and control cats ($P < 0.003$). S100A12 concentrations in CE cats (median 94 ng/g; IQR 16–548) were statistically significantly higher compared with control cats ($P < 0.001$). The area under the receiver operating characteristic curve (AUROC) to separate healthy cats from CE cats was 0.81 (95% confidence interval [CI] 0.70–0.92) and was statistically significant ($P < 0.001$). The AUROC to separate cats with IBD from cats with LSA was 0.51 (95% CI 0.34–0.68) and was not statistically significant ($P = 0.9$).

Conclusions and relevance Fecal S100A12 concentrations at the time of diagnostic investigation were higher in cats with CIE and LSA than in healthy controls but did not differ between cats with LSA and those with CIE/IBD. This study is an initial step toward evaluating a novel non-invasive marker of feline CIE. Further studies are needed to determine the diagnostic utility of fecal S100A12 concentrations in cats with CE, including comparing cats with IBD/CIE and LSA, and to compare them with cats with extra-gastrointestinal disease.

Keywords: Biomarker; gastrointestinal; inflammation; inflammatory bowel disease; lymphoma

Accepted: 1 March 2023

Introduction

Chronic enteropathies (CE) are common in cats. Cats often present with clinical signs such as vomiting, diarrhea, anorexia, hyporexia and/or weight loss.[1–5] The diagnostic work-up for these patients involves a systematic evaluation to exclude secondary causes of gastrointestinal (GI) signs, and documentation of mucosal inflammation based on histopathology after biopsy.[3–5] Histopathology aims to distinguish between inflammatory bowel disease (IBD) or chronic inflammatory enteropathy (CIE)

[1]Internal Medicine Department, Schwarzman Animal Medical Center, New York, NY, USA
[2]Gastrointestinal Laboratory, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Corresponding author:
Kailee Zornow DVM, Internal Medicine Department, Schwarzman Animal Medical Center, 510 E 62nd St, New York, NY 10065, USA
Email: kailee.zornow@amcny.org

and alimentary lymphoma (LSA); however, changes in mucosal architecture are not always definitive. Additional testing with immunohistochemistry (IHC) and molecular clonality testing with PCR are often used to help distinguish between IBD and LSA.[6–8]

The pathophysiology of inflammatory enteropathies is not fully understood, but has been hypothesized to involve interactions between environmental, genetic and immune factors, leading to chronic inflammation.[3–5,9] In humans and companion animals, mutations in innate immune receptors, upregulated epithelial major histocompatibility complex (MHC) class II molecule expression, microbiota alterations and increased antibody reactivity to antigens of commensal microbiota have been linked to CE.[9–15] Additionally, dietary factors are hypothesized to play a role, based on a clinical response to dietary trials in both dogs and cats.[2,16–19]

S100A12 (calgranulin C) is a damage-associated molecular pattern molecule of the innate immune response and calcium-binding protein of the S100/calgranulin family.[20,21] It is predominantly released from activated polymorphonuclear cells and targets several different receptor proteins, including receptors for activated glycation end products (RAGE).[21,22] The ligand–RAGE interaction activates nuclear factor kappa B, leading to the production of proinflammatory cytokines and chemokines.[22,23] S100A12 plays a central role in both innate and acquired immune responses and has several cellular regulatory functions.[20,21,24] It is a sensitive and specific marker of localized inflammatory processes and has been associated with acute inflammation, chronic inflammation and malignancy in humans.[25–33] S100A12 accumulates at sites of inflammation and is stable in feces.[29,34] Fecal S100A12 is a biomarker of intestinal inflammation in dogs with CE and has been associated with the severity of endoscopic lesions and response to treatment.[35–38]

Biomarkers of inflammation are considered valuable for the diagnosis and monitoring of IBD in humans.[25–32,39] S100A12 is linked to many human inflammatory GI diseases.[27–33] S100A12 and other minimally invasive biomarkers for CE diagnosis, disease monitoring, and monitoring response to therapy have been evaluated in dogs.[35–38,40–42] Numerous biomarkers have been evaluated in cats with CE, including cobalamin, folate, albumin, serum amyloid A, serum haptoglobin, calprotectin and the diagnostic marker of ultrasonographic mucosal thickening, though histopathology remains the gold standard for a diagnosis of CIE.[43–47] There is a paucity of sensitive and specific non-invasive biomarkers of GI inflammation in cats, with a recent focus on the S100/calgranulin family of proteins.

To our knowledge, there have been no published reports of fecal S100A12 concentrations in cats diagnosed with CE. The aim of this initial study was to quantify and compare fecal S100A12 concentrations in cats diagnosed with CE and healthy control cats. We propose that cats with CIE/IBD and LSA have higher S100A12 concentrations than control cats.

Materials and methods

Ethics approval

The prospective, cross-sectional study design was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC number 11-25-20) at the Schwarzman Animal Medical Center. An informed consent form was provided to the owner of each cat prior to inclusion in the study.

Sampling population

Cats with CE aged >1 year (n = 56) presenting for routine diagnostic evaluation of chronic (⩾3 weeks' duration) signs of GI disease (ie, vomiting, diarrhea, hyporexia or anorexia, abdominal pain and weight loss), including a planned endoscopic collection of GI tissue biopsies according to World Small Animal Veterinary Association (WSAVA) guidelines, were prospectively enrolled.[48] All cats underwent a routine diagnostic evaluation, including a complete blood count, serum chemistry profile, urinalysis, abdominal ultrasound imaging and additional diagnostics to rule out other causes of chronic GI signs (eg, fecal testing, serum trypsin-like immunoreactivity, feline pancreas-specific lipase, cobalamin and folate) at the discretion of the attending clinician at the Schwarzman Animal Medical Center. The severity of the cat's clinical signs at presentation was quantified by a single investigator using the feline CE activity index (FCEAI) scoring system.[49] Treatment of each individual cat, including dietary management, antibiotic treatment, supplements, anti-inflammatory or immunosuppressant therapy, was also at the discretion of the attending clinician. Exclusion criteria applied retrospectively included an unclear histopathologic diagnosis of IBD vs LSA, normal histopathology or diagnosis of neoplasia other than LSA (Figure 1).

Healthy control cats (n = 19) aged >1 year with no history of GI signs were included. Control cats were not on any medications for at least 30 days prior to sample collection. Dietary history was collected, and control cats were excluded if fed a raw diet.

Sample collection

Fresh fecal samples (~1 g) were collected from affected cats with gentle lubricated digital removal while under anesthesia and prior to bowel preparation at the time of endoscopy. Spontaneously voided fecal samples were provided by owners of healthy control cats in at-home litterboxes. Control fecal samples collected at home were refrigerated (at approximately 3°C) and provided within 24 h of defecation. Each fecal sample was

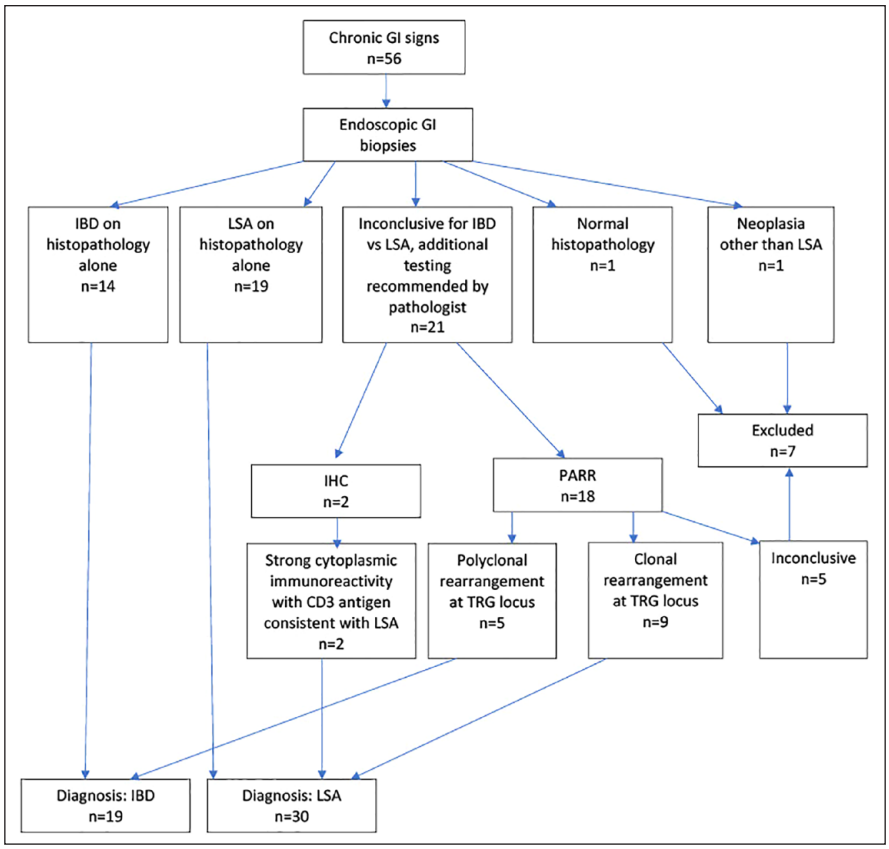

Figure 1 Diagnostic evaluation of eligible affected cats. Fifty-six cats with chronic gastrointestinal (GI) signs were prospectively enrolled. Seven cats were excluded due to unclear histopathologic diagnosis of inflammatory bowel disease (IBD) vs alimentary lymphoma (LSA; n = 5), normal histopathology (n = 1) or a diagnosis of neoplasia other than LSA (n = 1) for a final affected study population of 49 cats. The IBD group consisted of 19 cats: 14 cats diagnosed with IBD based on histopathology alone and five with PCR for antigen receptor rearrangement (PARR) for the T-cell receptor gamma (TRG) locus contribution. The LSA group consisted of 30 cats: 19 cats diagnosed with LSA based on histopathology alone, two with immunohistochemistry (IHC) contribution and nine with PARR contribution

immediately frozen (–80°C) until batch stool shipment to the Gastrointestinal Laboratory at Texas A&M University (TAMU).

Endoscopic GI tissue biopsies were obtained from each cat with the extent of biopsy acquisition (upper vs upper and lower endoscopy with biopsies) left to the discretion of the attending clinician. Endoscopic evaluation was performed using either a 103 cm endoscope with an 8.6 mm outside diameter and a 2.8 mm working channel (GIF-160; Olympus), or a 140 cm endoscope with a 7.9 mm outside diameter and a 2.8 mm working channel (60714 NKSK; Karl Storz). All biopsies were obtained using 2.8 mm cup biopsy forceps. All tissue biopsies were immediately fixed in 10% formalin and transported to the laboratory for histopathologic evaluation.

Sample analyses

Histopathologic evaluation of GI tissue biopsies was performed by one of 10 board-certified veterinary pathologists at IDEXX Reference Laboratories. A diagnosis of IBD was based on a main histologic finding of diffuse lymphoplasmacytic inflammatory infiltrate, with severity of inflammation based on the reported WSAVA histopathological standards.[50] A diagnosis of lymphoma was based on an expanded monomorphic population of lymphocytes obscuring architecture with fusion of villous papillae, and the presence of intraepithelial lymphocytes forming discrete plaques, nests or clusters.[51] In cases where histopathology was insufficient to differentiate IBD from LSA, additional diagnostic modalities were employed. Immunohistochemical staining was performed and interpreted by the same veterinary pathologist who interpreted the histopathology at IDEXX Reference Laboratories. PCR for antigen receptor rearrangement for the T-cell receptor gamma locus (PARR) was performed by a single board-certified veterinary pathologist at the Animal Health Laboratory at the University of Guelph, Ontario. IHC demonstrating strong cytoplasmic immunoreactivity with CD3 antigen, or PARR showing clonality combined with clinical, laboratory and histopathologic findings, was considered consistent with a diagnosis of lymphoma.[8,52]

Fecal S100A12 concentrations were measured at the Gastrointestinal Laboratory at TAMU using an analytically validated in-house ELISA.[34,53–55] A canine S100A12-ELISA was first developed and shown to be accurate, reproducible, and sufficiently linear and precise for the measurement of S100A12 in cat serum and fecal samples.[55] A reference interval (RI) for feline fecal S100A12 was reported in a previous study.[55] However, since this RI has not been updated and the assay is only used infrequently, a statistical comparison between groups was performed rather than comparing results to the previously reported RI.

Data analyses

A commercially available statistical software package was used for all statistical analyses (SAS version 9.4, 2014). The normality of error residuals was analyzed by the Kolmogorov–Smirnov test and visual inspection. Descriptive statistics were used to analyze continuous variables and reported as median and interquartile range (IQR). Categorical data are presented as counts and percentages. A Kruskal–Wallis test was used to compare S100A12 concentrations between cats with IBD and healthy controls, cats with LSA and healthy controls, a combined CE group and healthy controls, and cats with IBD and LSA. Receiver operating characteristic (ROC) curve analysis was used to calculate the area under the ROC (AUROC) curve and served to calculate sensitivity and specificity at cutoff concentrations (determined by the Youden index) to differentiate (1) cats with CE from healthy cats and (2) cats with IBD from cats with LSA. Spearman's rank-order correlation coefficient (r) was used to evaluate the correlation between S100A12 and other variables, including age and FCEAI score. A nonparametric Wilcoxon rank-sum test was used to compare S100A12 concentrations between cats with and without hyperthyroidism, and between cats with and without chronic kidney disease (CKD). Post-hoc Dunn's multiple comparisons test was used to identify differences in S100A12 concentrations between healthy cats, CE cats with CKD and CE cats without CKD. Statistical significance was set at $P < 0.05$.

Results

Study population

A total of 56 cats with CE were evaluated for enrollment into the study. Seven cats were excluded due to an unclear histopathologic diagnosis of IBD vs LSA (n = 5), normal histopathology (n = 1) or a diagnosis of neoplasia other than LSA (n = 1) for a final affected study population of 49 cats (median age 12 years [range 4–20]; 28 neutered males and 21 spayed females; median FCEAI score 6) (Figure 1). The most common presenting complaints included vomiting, weight loss, hyporexia, diarrhea, lethargy, hiding, hematochezia, regurgitation and constipation. Nineteen healthy control cats (median age 7 years [range 1–13]; 12 neutered males and 7 spayed females) were also enrolled into the study. The characteristics of each study population, including sex, age, breed, FCEAI score and most common presenting complaints are summarized in Table 1.

Of the 49 cats with CE, 21 had biopsies performed of the stomach/duodenum alone, 10 had biopsies performed of the stomach/duodenum and colon, 16 had biopsies performed of the stomach/duodenum, ileum and colon, and two had biopsies performed of the colon only. Thirty cats were diagnosed with LSA (median age 12 years; 17 male neutered and 13 female spayed; median FCEAI score 6) and 19 were diagnosed with IBD (median age 10 years; 11 male neutered and eight female spayed; median FCEAI score 5.5) (Figure 1). Nineteen cats were diagnosed with LSA based on histopathology alone, nine with PARR contribution to diagnosis and two with IHC confirmation. Twenty-eight of 30 cats were diagnosed with epitheliotropic small-cell lymphoma. One of the cats with small-cell lymphoma was diagnosed concurrently with B-cell lymphoma of the stomach. Of the two cats whose histopathology was not small-cell lymphoma, one was diagnosed with intermediate-to-large-cell lymphoma without epitheliotropism, and the other had a neoplastic population of small-to-intermediate-sized lymphocytes, raising concern for large granular lymphoma. Of the 19 cats diagnosed with IBD, PARR was performed in five cases due to features consistent with LSA, but the PARR results were polyclonal and most consistent with inflammation. A total of three cats with IBD had ileal biopsies performed. Blind biopsies were acquired in two cats, and one cat had successful ileal intubation for biopsy acquisition. Thirteen LSA cats had ileal biopsies performed; blind biopsies were acquired in six cats, and seven cats had visualized ileal biopsies after successful intubation. Ten of these 13 cats had evidence of LSA in the ileum; however, all 13 cats had evidence of LSA on duodenal histopathology. All colonic histopathology showed inflammatory changes consistent with IBD. The location of biopsy acquisition and severity of lymphoplasmacytic histopathologic inflammation in the LSA and IBD populations is summarized in Table 2.

Six CE cats received glucocorticoids prior to diagnostic evaluation (prednisolone, n = 5; inhaled, fluticasone, n = 1). A total of three cats were receiving oral metronidazole at the time of endoscopy. One cat had received a subcutaneous injection of a long-acting antibiotic (cefovecin) 5 days prior for a dental procedure. Eleven cats were trialed on a hydrolyzed or novel protein diet trial with persistence of GI signs. One CE cat was being fed a raw diet. CE cats were not excluded for other comorbid conditions, although 17 cats had CKD and five had hyperthyroidism. Of the cats with CKD, 16/17 were of International Renal Interest Society (IRIS) stage 1 or 2;

Table 1 Summary of the study populations

	Total CE population (n = 49)	IBD (n = 19)	LSA (n = 30)	Control (n = 19)
Sex	28 MN + 21 FS	11 MN + 8 FS	17 MN + 13 FS	12 MN + 7 FS
Median (IQR) age (years)	12 (8.25–14.5)	10 (7–14)	12 (10–15)	7 (4–10)
Breed (n)	DSH (34); DLH (3); Abyssinian (2); Ragdoll (2); American Shorthair (1); Bengal (1); Birman (1); Burmese (1); DMH (1); Oriental Shorthair (1); Siamese (1); Tonkinese (1)	DSH (13); DMH (1); DLH (1); Birman (1); Ragdoll (1); Siamese (1); Tonkinese (1)	DSH (21); DLH (2); Abyssinian (2); American Shorthair (1); Bengal (1); Burmese (1); Oriental Shorthair (1); Ragdoll (1)	DSH (18); DMH (1)
Median (IQR) FCEAI score	6 (5–7)	5.5 (5–7)	6 (5–8)	NA
Most common presenting complaint, n (%)	Vomiting, 32 (65); weight loss, 29 (59); hyporexia, 20 (41); diarrhea, 16 (32); lethargy/hiding, 7 (14); hematochezia, 4 (8); regurgitation, 1 (2); constipation, 1 (2)	Vomiting, 9 (47); weight loss, 10 (52); hyporexia, 6 (32); diarrhea, 6 (32); lethargy/hiding, 5 (26); hematochezia, 2 (11); regurgitation, 0 (0); constipation, 1 (5)	Vomiting, 23 (77); weight loss, 19 (63); hyporexia, 14 (47); diarrhea, 10 (33); lethargy/hiding, 2 (7); hematochezia, 2 (7); regurgitation, 1 (3); constipation, 0 (0)	NA

A feline CE activity index (FCEAI) score could not be calculated for one cat with chronic enteropathy (CE) in the inflammatory bowel disease (IBD) group due to lack of applicable history
LSA = alimentary lymphoma; MN = male neutered; FS = female spayed; IQR = interquartile range; DSH = domestic shorthair; DLH = domestic longhair; DMH = domestic mediumhair; NA = not applicable

Table 2 Summary of endoscopic gastrointestinal tissue biopsy acquisition for cats with inflammatory bowel disease (IBD) and alimentary lymphoma (LSA)

	LSA (n = 30)	Severity	IBD (n = 19)	Severity	Total (n = 49)
Stomach/duodenum	13	Mild: 2; moderate: 9; severe: 2	8	Mild: 2; moderate: 6; severe: 0	21
Stomach/duodenum/colon	4	Mild: 1; moderate: 3; severe: 0	6	Mild: 0; moderate: 4; severe: 2	10
Stomach/duodenum/ileum/colon	13	Mild: 0; moderate: 8; severe: 5	3	Mild: 0; moderate: 3; severe: 0	16
Colon only	0	Not available	2	Mild: 0; moderate: 2; severe: 0	2

The extent of tissue acquisition was left to the discretion of the attending clinician. Overall histopathologic severity of lymphoplasmacytic inflammation per site is also summarized based on the microscopic description. In cases with mixed severity of inflammation, the most severe degree of inflammation noted in the histopathology report was assigned

1/17 cats had IRIS stage 3 CKD. All cats with hyperthyroidism were well controlled on medical management at the time of investigation.

Fecal S100A12 concentrations

Fecal S100A12 concentrations ranged from <1 ng/g to >81,241 ng/g in individual fecal samples from all cats diagnosed with CE (median 94; IQR 16–548). In cats diagnosed with IBD, fecal S100A12 concentrations ranged from <1 ng/g to 57,987 ng/g (median; IQR 15–973). In cats diagnosed with LSA, fecal S100A12 concentrations ranged from 3 ng/g to >81,242 ng/g (median 110; IQR 18–548). Fecal S100A12 concentrations in the control group ranged from 1 ng/g to 223 ng/g (median; IQR 2–25) (Figure 2).

Fecal S100A12 concentrations were statistically significantly higher in cats diagnosed with CE (combined IBD/LSA, median 94 ng/g; IQR 16–548) compared with healthy control cats (median 4 ng/g; IQR 2–25 [P <0.001]). Fecal S100A12 concentrations were statistically significantly

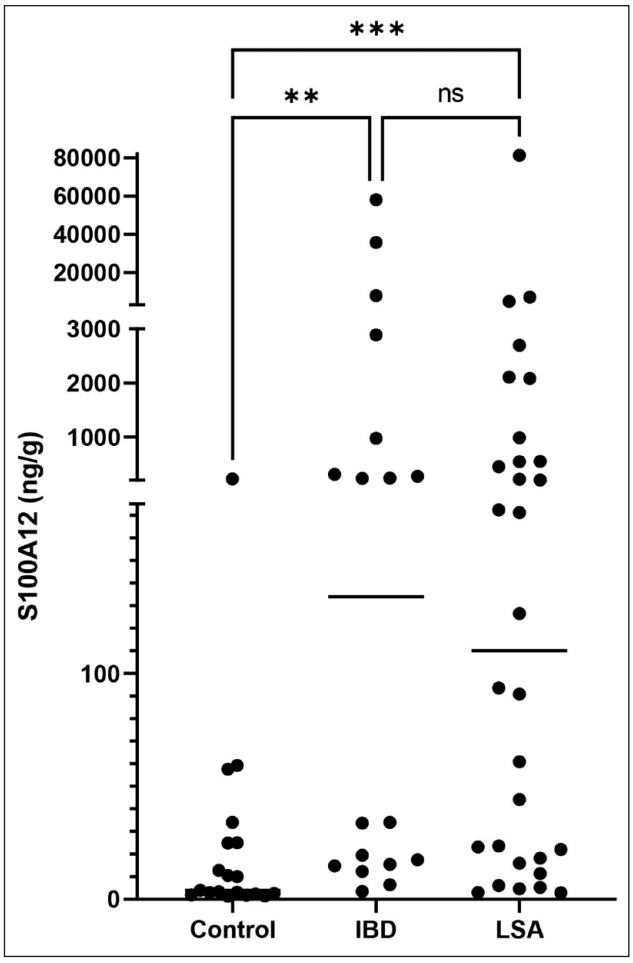

Figure 2 Fecal S100A12 concentrations (ng/g) in control cats, cats with inflammatory bowel disease (IBD) and cats with alimentary lymphoma (LSA). In the IBD group, fecal S100A12 concentrations ranged from <1 ng/g to 57,987 ng/g (median 34; interquartile range [IQR] 15–973). In the LSA group, fecal S100A12 concentrations ranged from 3 ng/g to >81,242 ng/g (median 110; IQR 18–548). Fecal S100A12 concentrations in the control group ranged from 1 ng/g to 223 ng/g (median 4; IQR: 2–25). Fecal S100A12 levels were statistically significantly higher in cats diagnosed with IBD compared with healthy controls (**P <0.003) and in cats diagnosed with LSA compared with healthy controls (***P <0.001). There was no statistically significant difference in fecal S100A12 concentrations in cats with LSA compared with those with IBD (P = 0.9; not significant [ns])

higher in cats diagnosed with IBD compared with healthy control cats (P <0.003) and in cats diagnosed with LSA compared with healthy control cats (P <0.001). There was no statistically significant difference in fecal S100A12 concentrations between cats with LSA and those with IBD (P = 0.9).

ROC analysis

The AUROC to separate healthy from CE cats was 0.81 (95% confidence interval [CI] 0.70–0.92) and was statistically significant (P <0.001; Figure 3a). When the cutoff value of S100A12 concentration between CE and healthy cats was set at 13.8 ng/g, the sensitivity was 79.6% (95% CI 66.3–88.5) and specificity was 68.4% (95% CI 46.0–84.6) for differentiating between the two groups. The AUROC to separate cats with IBD from cats with LSA was 0.51 (95% CI 0.34–0.68) and was not statistically significant (P = 0.9; Figure 3b). When the cutoff value of S100A12 concentration between IBD and LSA cats was set as 39.1 ng/g, the sensitivity was 63.3% (95% CI 45.5–78.1) and specificity was 52.6% (95% CI 31.7–72.7) for differentiating between the two groups.

Other variables

There was no correlation between the age of all the cats and S100A12 concentration (r = 0.085, P = 0.49), or between the age of cats with CE and S100A2 concentration (r = –0.031, P = 0.83). There was also no correlation between FCEAI score and S100A12 concentration (r = –0.056, P = 0.71). There was no statistically significant difference in S100A12 concentration between cats with CE with hyperthyroidism (n = 5) and cats without hyperthyroidism (n = 63; P = 0.64). There was a statistically significant difference in S100A12 concentrations between CE cats with CKD (n = 17) and all cats without CKD (n = 51; P <0.003). However, while there was a statistically significant difference in S100A12 concentrations between control cats and CE cats without CKD (P <0.006) and control cats and CE cats with CKD (P <0.001), there was no statistically significant difference in S100A12 concentration between CE cats without CKD and CE cats with CKD (P = 0.34).

Discussion

The purpose of this study was to quantify and compare fecal S100A12 concentrations in cats with CE to healthy control cats. To our knowledge, this is the first study to evaluate fecal S100A12 in cats with CE. A diagnosis of IBD/CIE and lymphoma requires evaluation of biopsy specimens in combination with clinical signs, dietary trials and assessment of clinical response.[48] However, GI biopsy acquisition is not appropriate or feasible for every cat with chronic GI signs. Factors taken into consideration include anesthetic concerns, client expectations, financial constraints and the limitations of an endoscopic procedure vs the invasiveness of a surgical procedure. For cases in which endoscopy is not pursued, clinically useful non-invasive biomarkers indicating GI inflammation may be helpful to raise the index of suspicion for underlying GI disease and guide empiric therapy. S100A12 is an inflammatory biomarker that can be measured in fecal samples.[27,29,31,32,35] Collection of feces for measurement of S100A12 is non-invasive and can be done at home by the owner, making this a user-friendly diagnostic tool.[56]

This study demonstrates that fecal S100A12 was significantly higher at the time of diagnostic investigation in cats with CE compared with healthy controls, with

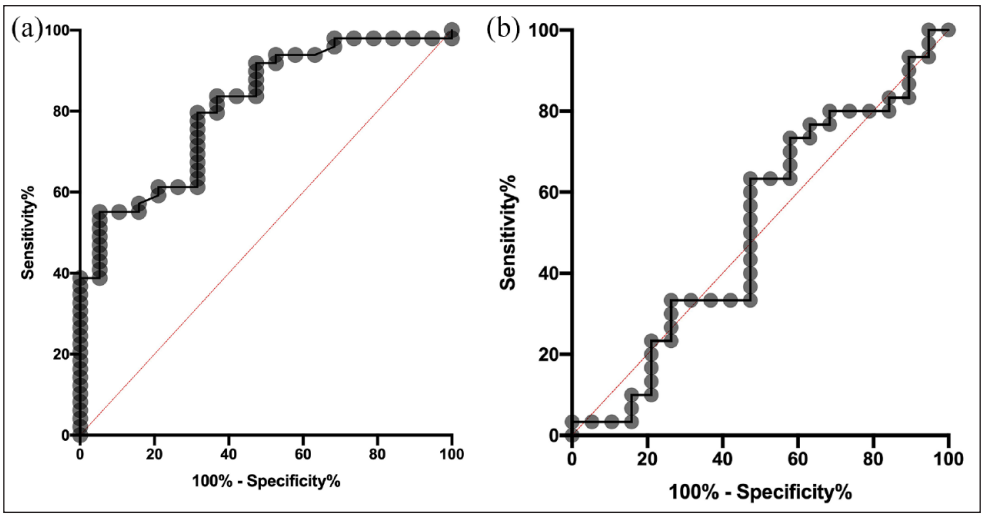

Figure 3 (a) Receiver operating characteristic (ROC) curve of fecal S100A12 concentration in healthy vs cats with chronic enteropathy. The area under the ROC (AUROC) curve is 0.81 (95% confidence interval [CI] 0.70–0.92; $P < 0.001$). (b) ROC curve of fecal S100A12 concentration for cats with inflammatory bowel disease vs those with alimentary lymphoma. The AUROC curve is 0.51 (95% CI 0.34–0.68; $P = 0.9$)

significant discrimination based on AUROC. Though we studied cats and recognize differences in disease processes, the results are in alignment with former studies performed in dogs and humans that showed concentrations of fecal canine S100A12 to be significantly higher in dogs with IBD compared with healthy controls, and a sensitive marker of GI inflammation with exceptional specificity in distinguishing between children with IBD and children without IBD.[29,31,36] The present study represents a first step toward developing a novel non-invasive marker of feline CIE as part of a multimodal approach to diagnosis of GI disease.

A secondary aim of the study was to compare fecal S100A12 concentrations in cats with IBD and LSA. The etiopathogenesis of feline low-grade LSA is not yet fully elucidated, but some cases are thought to arise from chronic untreated GI inflammation and neoplastic transformation of IBD to LSA over time.[6,43] In the present study, fecal S100A12 concentrations did not significantly differ between cats with IBD and LSA. There was no discrimination between IBD and LSA groups based on AUROC, and possible cutoffs had low sensitivities and specificities. A recent study that evaluated IHC for S100A8/A9 and S100A12 in GI biopsies of cats with CE showed slightly higher GI mucosal S100A8/A9 and S100A12 cell counts than controls, but there was no significant difference between cats with IBD and LSA.[57] The lack of differentiation between IBD and LSA aligns with the current challenges in veterinary medicine in distinguishing between the two disease processes; as stated earlier, differentiation between IBD and LSA is not always definitive with a single diagnostic test such as histopathology alone. Similarly, fecal S100A12 concentrations did not conclusively distinguish between the two disease entities in this cohort of cats.

In this study, there was no correlation between clinical disease activity (FCEAI score) in the CE group and fecal S100A12 concentration. FCEAI score is calculated by tallying the presence of and severity of GI signs (ie, appetite, vomiting, diarrhea, weight loss and attitude/activity), the presence of endoscopic lesions and changes in biochemical parameters, including total protein, alanine transaminase/alkaline phosphatase and phosphorous.[48] Cats with CE can present in a variety of different ways, leading to a varying degree of severity of FECAI score. Some cats may only present with weight loss, despite a good appetite, energy level and normal biochemical parameters, leading to a low FCEAI score. In contrast, cats may have severe GI signs and biochemical changes, leading to high FCEAI scores. Lack of correlation between FCEAI score in the affected cats and fecal S100A12 concentration is likely explained by the diverse clinical presentation of cats with CE.

In dogs, cutoff values for fecal S100A12 have been shown to distinguish between dogs requiring anti-inflammatory or immunosuppressive treatment and dogs with food-responsive or antibiotic-responsive enteropathies ($\geq$490 ng/g; sensitivity 64%, specificity 77%).[37] A study in dogs also showed that fecal S100A12 concentrations >2700 ng/g distinguished between dogs with CE refractory to treatment and those with partial remission.[37] In our cohort of cats, a cutoff value of 13.8 ng/g distinguished between cats with CE and control cats with a sensitivity of 79.6% and specificity of 68.4%. Given that there was no significant differentiation between fecal S100A12 concentrations in IBD and LSA cats based on AUROC

analysis, cutoff values in the two groups were not reliable and had a low sensitivity and specificity. For example, a cutoff of 39.1 ng/g distinguishes between cats with LSA and IBD with a sensitivity of 63.3% and a specificity of 52.6%. In this cohort of cats, we were unable to establish a statistically significant cutoff between IBD and LSA cats.

The median age of cats in the CE and control groups differed (12 years vs 7 years); however, a recent study did not find an association between S100A12 concentrations and age in healthy puppies and adult dogs.[42] In our study, there was no correlation between age and S100A12 concentration in all cats, or between age and S100A12 concentration in CE cats. The presence of controlled hyperthyroidism did not significantly affect fecal S100A12 concentration. Interestingly, the presence of CKD resulted in a significantly higher fecal S100A12 concentration compared with cats without CKD. However, all cats with CKD were in the CE group. Cats with CE without CKD still had significantly higher fecal S100A12 concentrations compared with healthy controls, and there was no significant difference in fecal S100A12 concentration between CE cats with CKD and CE cats without CKD. Further studies are needed to evaluate for an individual effect of CKD on fecal S100A12 concentration.

There were a few outliers in the CE group, with fecal S100A12 concentrations >35,000 ng/g. The largest concentration was >81,241 ng/g in a cat in the LSA group. Two cats in the IBD group had fecal S100A12 concentrations of 57,986 ng/g and 35,215 ng/g, respectively. Two of these outliers had large bowel signs as part of their presenting complaints and had evidence of colitis on histopathology. The third outlier (fecal concentration 35,215 ng/g) did not have large bowel signs and the colon was not included in endoscopic evaluation. In the absence of other explanations for these outliers, it is possible that colitis may have a more profound effect on fecal S100A12 concentrations, though further studies are needed for investigation.

This study had several limitations. Fecal S100A12 concentrations were quantified using a single fecal sample, whereas prior studies have suggested and used fecal samples collected on three consecutive days for analysis, owing to intra-individual variability.[53] Validation of the canine S100A12 for use in cats found a low level of short-term biologic variation in fecal samples.[55] Thus, using fecal samples from three consecutive days would be preferred but was determined not to be feasible for this study based on owner compliance to stool acquisition.[55] Collection of the stool samples in the CE group was performed by gentle lubricated digital removal under anesthesia prior to bowel preparation. As S100A12 concentrations are reflective of bowel granulocytes, the method of stool collection may have affected the results, though no studies have been performed to evaluate this in cats. Additionally, stool samples for all cats were stored in –80°C freezers and sent as a batch for analysis. CE group samples were immediately frozen, and healthy control samples were frozen within 24 h of collection. The length of storage may have affected S100A12 concentrations, thus lowering the values, but no study on the impact of feline fecal S100A12 concentrations has been done to date, although S100A12 has been demonstrated to be stable in fecal samples at various temperatures for 7 days in canine studies and up to 6 months in human studies.[29,34,36,56]

Several cats with CE were receiving medications at the time of sample collection, including corticosteroids (n = 6) or antibiotics (n = 4). A previous study showed that corticosteroid treatment did not have a significant effect on canine serum S100A12 concentrations.[24] However, the effect of corticosteroid therapy on fecal S100A12 in cats and possible effects of antibiotics on fecal S100A12 concentrations are unknown. Ideally, all cats enrolled would have been free of any medications prior to endoscopy and stool collection; however, this cohort of cats represents a typical patient population. Additionally, the diet of enrolled cats was not standardized, and diet recommendations were clinician and owner dependent. We relied on observed health of control cats and provided owner and veterinary record history, rather than a full work-up to ensure systemic health. Lastly, the healthy control group sample size was smaller than the total affected population sample size, and controls were not breed or age matched to affected cats. This may have affected the results, though most cats in all groups were neutered/spayed, domestic shorthair, domestic mediumhair or domestic longhair, and there was no correlation between the age of all cats and S100A12 concentration.

Alimentary LSA may affect any section of the GI tract; however, the most common sites include the jejunum, followed by the ileum and duodenum.[51,58] Not every CE cat had a full endoscopic evaluation, including biopsy of the duodenum, jejunum, ileum and colon. While 13 cats with LSA had biopsies performed of the ileum, only three cats with IBD had ileal biopsies performed. While all LSA cats with ileal biopsies also had evidence of small-cell lymphoma in the duodenum, it is possible that some cats with IBD may have had undiagnosed LSA without full endoscopic evaluation. This could have contributed to the lack of statistically significant difference in fecal S100A12 concentrations between the two CE groups. Interestingly, all colonic histopathology was consistent with colitis, regardless of the presence of IBD or LSA in other biopsy sites. This aligns with a previous study demonstrating the presence of LSA in only 1/5 colonic biopsies.[58]

This study provides important initial data supporting future studies evaluating fecal S100A12 as a novel non-invasive biomarker of feline inflammatory CE. Further research with the establishment of RIs, multiple daily stool samples, pre- and post-therapy measurements, and larger-scale studies comparing cats with IBD to LSA may

help determine whether this biomarker might be useful for the diagnosis of disease or monitoring response to therapy.

Conclusions

Fecal S100A12 concentrations are increased in cats with CE, and should be further evaluated as a biomarker to identify cats with GI inflammation and its severity. However, in this population of cats, fecal S100A12 did not distinguish between cats with IBD or LSA.

Author note Part of this material was presented as an abstract at the 2021 ECVIM-CA congress via a virtual poster presentation.

Conflict of interest JA Lidbury, JS Suchodolski and JM Steiner are employees of the Gastrointestinal Laboratory at Texas A&M University, which offers diagnostic testing, including fecal S100A12 measurement, on a fee-for-service basis.

Funding The authors received no financial support for the research, authorship, and/or publication of this article.

Ethical approval The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognised high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed consent Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

ORCID iD Kailee A Zornow https://orcid.org/0000-0001-7950-4938
Jennifer E Slovak https://orcid.org/0000-0001-8516-2380
Joerg M Steiner https://orcid.org/0000-0003-3336-2086

References

1 Verlinden A, Hesta M, Millet S, et al. **Food allergy in dogs and cats: a review.** *Crit Rev Food Sci Nutr* 2006; 46: 259–273.
2 Guilford WG, Jones BR, Markwell PJ, et al. **Food sensitivity in cats with chronic idiopathic gastrointestinal problems.** *J Vet Intern Med* 2001; 15: 7–13.
3 Jergens AE. **Feline idiopathic inflammatory bowel disease: what we know and what remains to be unraveled.** *J Feline Med Surg* 2012; 14: 445–458.
4 de Rezende CE and Al-Ghazlat S. **Feline small cell lymphosarcoma versus inflammatory bowel disease: treatment and prognosis.** *Compend Contin Educ Vet* 2013; 35: E1–E6.
5 Jergens A and Simpson KW. **Inflammatory bowel disease in veterinary medicine.** *Front Biosci (Elite Ed)* 2012; 4: 1404–1419.
6 Marsilio S. **Differentiating inflammatory bowel disease from alimentary lymphoma in cats: does it matter?** *Vet Clin Small Anim* 2021; 51: 93–109.
7 Sabattini S, Bottero E, Turba ME, et al. **Differentiating feline inflammatory bowel disease from alimentary lymphoma in duodenal endoscopic biopsies.** *J Small Anim Pract* 2016; 57: 396–401.
8 Moore PF, Woo JC, Vernau W, et al. **Characterization of feline T cell receptor gamma (TCRG) variable region genes for the molecular diagnosis of feline intestinal T cell lymphoma.** *Vet Immunol Immunopathol* 2005; 106: 167–178.
9 Packey CD and Sartor RB. **Commensal bacteria, traditional and opportunistic pathogens, dysbiosis and bacterial killing in inflammatory bowel diseases.** *Curr Opin Infect Dis* 2009; 22: 292–301.
10 Xavier RJ and Podolsky DK. **Unraveling the pathogenesis of inflammatory bowel disease.** *Nature* 2007; 448: 427–434.
11 Kathrani A, House A, Catchpole B, et al. **Polymorphisms in the *TLR4* and *TLR5* gene are significantly associated with inflammatory bowel disease in German Shepherd dogs.** *PLoS One* 2010; 5. DOI: 10.1371/journal.pone.0015740.
12 Mayer L, Eisenhardt D, Salomon P, et al. **Expression of class II molecules on intestinal epithelial cells in humans. Differences between normal and inflammatory bowel disease.** *Gastroenterology* 1991; 100: 3–12.
13 Koretz K, Momburg F, Otto HF, et al. **Sequential induction of MHC antigens on autochthonous cells of ileum affected by Crohn's disease.** *Am J Pathol* 1987; 129: 493–502.
14 Waly NE, Stokes CR, Gruffydd-Jones TJ, et al. **Immune cell populations in the duodenal mucosa of cats with inflammatory bowel disease.** *J Vet Intern Med* 2004; 18: 816–825.
15 Honneffer JB, Minamoto Y and Suchodolski JS. **Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs.** *World J Gastroenterol* 2014; 20: 16489–16497.
16 Mandigers PJ, Biourge V and Van Den Ingh TS. **A randomized, open-label, positively-controlled field trial of a hydrolyzed protein diet in dogs with chronic small bowel enteropathy.** *J Vet Intern Med* 2010; 24: 1350–1357.
17 Laflamme DP, Xu H and Long GM. **Effects of diets differing in fat content on chronic diarrhea in cats.** *J Vet Intern Med* 2011; 25: 230–235.
18 Rudinsky AJ, Howard JP, Bishop MA, et al. **Dietary management of presumptive protein-losing enteropathy in Yorkshire Terriers.** *J Small Anim Pract* 2017; 59: 103–108.
19 Waly NE, Biourge V, Day MJ, et al. **Use of a hydrolysed soya isolate-based diet in the management of chronic idiopathic inflammatory bowel disease and dietary hypersensitivity in cats.** *Assiut Vet Med J* 2010; 56: 158–169.
20 Dell'Angelica EC, Schleicher CH and Santome JA. **Primary structure and binding properties of calgranulin C, a novel S100-like calcium-binding protein from pig granulocytes.** *J Biol Chem* 1994; 269: 28929–28936.
21 Vogl T, Propper C and Hartmann M. **S100A12 is expressed exclusively by granulocytes and acts independently from MRP8 and MRP14.** *J Biol Chem* 1999; 274: 25291–25296.

22 Hatakeyama T, Okata M, Shimamoto S, et al. **Identification of intracellular target proteins of the calcium-signaling protein S100A12.** *Eur J Biochem* 2004; 271: 3765–3775.

23 Luckschander N, Hall JA, Gaschen F, et al. **Activation of nuclear factor-kappaB in dogs with chronic enteropathies.** *Vet Immunol Immunopathol* 2010; 133: 228–236.

24 Goyette J and Geczy CL. **Inflammation-associated S100 proteins: new mechanisms that regulate function.** *Amino Acids* 2011; 41: 821–842.

25 Kryzstek-Korpacka M, Kempinski R, Bromke M, et al. **Biochemical biomarkers of mucosal healing for inflammatory bowel disease in adults.** *Diagnostics* 2020; 10: 367.

26 Siddiqui I, Majid H and Abid S. **Update on clinical and research application of fecal biomarkers for gastrointestinal diseases.** *World J Gastrointest Pharmacol Ther* 2017; 8: 39–46.

27 Kaiser T, Langhorst J, Wittkowski H, et al. **Faecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome.** *Gut* 2007; 56: 1706–1713.

28 Dabritz J, Langhorst J, Lugering A, et al. **Improving relapse prediction in inflammatory bowel disease by neutrophil-derived S100A12.** *Inflamm Bowel Dis* 2013: 19: 1130–1138.

29 de Jong NS, Leach ST and Day AS. **Fecal S100A12: a novel noninvasive marker in children with Crohn's disease.** *Inflamm Bowel Dis* 2006; 12: 566–572.

30 Manolakis AC, Kapsoritakis AN, Georgoulias P, et al. **Moderate performance of serum S100A12, in distinguishing inflammatory bowel disease from irritable bowel syndrome.** *BMC Gastroenterol* 2010; 10: 118. DOI: 10.1186/1471-230X-10-118.

31 Sidler MA, Leach ST and Day AS. **Fecal S100A12 and fecal calprotectin as noninvasive markers for inflammatory bowel disease in children.** *Inflamm Bowel Dis* 2008; 14: 359–366.

32 Foell D, Kucharzik T, Kraft M, et al. **Neutrophil derived human S100A12 (EN-RAGE) is strongly expressed during chronic active inflammatory bowel disease.** *Gut* 2003; 52: 847–853.

33 Carvalho A, Lu J, Francis JD, et al. **S100A12 in digestive diseases and health: a scoping review.** *Gastroenterol Res Pract* 2020; 2020. DOI: 10.1155/2020/2868373.

34 Heilmann RM, Cranford SM, Ambrus A, et al. **Validation of an enzyme-linked immunosorbent assay (ELISA) for the measurement of canine S100A12.** *Vet Clin Pathol* 2016; 45: 135–147.

35 Heilmann RM, Nestler J, Schwartz J, et al. **Mucosal expression of S100A12 (calgranulin C) and S100A8/A9 (calprotectin) and correlation with serum and fecal concentrations in dogs with chronic inflammatory enteropathy.** *Vet Immunol Immunopathol* 2019; 211: 64–74.

36 Heilmann RM, Aurelien G, Allenspach K, et al. **Association between fecal S100A12 concentration and histologic, endoscopic, and clinical disease severity in dogs with idiopathic inflammatory bowel disease.** *Vet Immunol Immunopathol* 2014; 158: 156–166.

37 Heilmann RM, Volkmann M, Otoni CC, et al. **Fecal S100A12 concentration predicts a lack of response to treatment in dogs affected with chronic enteropathy.** *Vet J* 2016; 215: 96–100.

38 Hanifeh M, Sankari S, Rajamaki MM, et al. **S100A12 concentrations and myeloperoxidase activities are increased in the intestinal mucosa of dogs with chronic enteropathy.** *BMC Vet Res* 2018; 14: 125. DOI: 10.1186/s12917-018-1441-0.

39 Sipponen T, Bjorkesten CGAF, Farkkila M, et al. **Faecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Crohn's disease treatment.** *Scand J Gastroenterol* 2010; 45: 325–331.

40 Otoni CC, Heilmann RM, Garcia-Sancho M, et al. **Serologic and fecal markers to predict response to induction therapy in dogs with idiopathic inflammatory bowel disease.** *J Vet Intern Med* 2018; 32: 999–1008.

41 Heilmann RM, Berghoff N, Mansell J, et al. **Association of fecal calprotectin concentrations with disease severity, response to treatment, and other biomarkers in dogs with chronic inflammatory enteropathies.** *J Vet Intern Med* 2018; 32: 679–692.

42 Heilmann RM, Grellet A, Grutzner N, et al. **Effect of selected gastrointestinal parasites and viral agents on fecal S100A12 concentrations in puppies as a potential comparative model.** *Parasit Vectors* 2018; 11: 252. DOI: 10.1186/s13071-018-2841-5.

43 Paulin MV, Couronne L, Beguin J, et al. **Feline low-grade alimentary lymphoma: an emerging entity and potential animal model for human disease.** *BMC Vet Res* 2018; 14: 306. DOI: 10.1186/s12917-018-1635-5.

44 Tamamoto T, Ohno K, Takahashi M, et al. **Serum amyloid A as a prognostic marker in cats with various diseases.** *J Vet Diagn* 2013; 25: 428–432.

45 Love EK, Leibman NF, Ringold R, et al. **Serum haptoglobin concentrations in feline inflammatory bowel disease and small-cell alimentary lymphoma: a potential biomarker for feline chronic enteropathies.** *J Feline Med Surg* 2021; 23: 959–964.

46 Heilmann RM, Grutzner N, Handl S, et al. **Preanalytical validation of an in-house radioimmunoassay for measuring calprotectin in feline specimens.** *Vet Clin Pathol* 2018; 47: 100–107.

47 Guttin T, Walsh A, Durham AC, et al. **Ability of ultrasonography to predict the presence and location of histologic lesions in the small intestine of cats.** *J Vet Intern Med* 2019; 33: 1278–1285.

48 Washabau RJ, Day MJ, Willard MD, et al. **Endoscopic, biopsy, and histopathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals.** *J Vet Intern Med* 2010; 24: 10–26.

49 Jergens AE, Crandell JM, Evans R, et al. **A clinical index for disease activity in cats with chronic enteropathy.** *J Vet Intern Med* 2010; 24: 1027–1033.

50 Day MJ, Bilzer T, Mansell J, et al. **Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group.** *J Comp Pathol* 2008; 138: S1–S43.

51 Kiupel M, Smedley RC, Pfent C, et al. **Diagnostic algorithm to differentiate lymphoma from inflammation in feline small intestinal biopsy samples.** *Vet Pathol* 2011; 48: 212–222.

52 Moore PF, Rodriguez-Bertos A and Kass PH. **Feline gastrointestinal lymphoma: mucosal architecture, immunophenotype, and molecular clonality.** *Vet Pathol* 2012; 49: 658–668.

53 Heilmann RM, Lanerie DJ, Ruaux CG, et al. **Development and analytic validation of an immunoassay for the quantification of canine S100A12 in serum and fecal samples and its biological variability in serum from healthy dogs.** *Vet Immunol Immunopathol* 2011; 144: 200–209.

54 Heilmann RM, Suchodolski JS and Steiner JM. **Purification and partial characterization of canine S100A12.** *Biochimie* 2010; 92: 1914–1922.

55 Bridges CS, Grutzner N, Suchodolski JS, et al. **Analytical validation of an enzyme-linked immunosorbent assay for the quantification of S100A12 in the serum and feces of cats.** *Vet Clin Pathol* 2019; 48: 754–761.

56 Heida A, Van de Vijver E, Kobold AM, et al. **Selecting children with suspected inflammatory bowel disease for endoscopy with the calgranulin C or calprotectin stool test: protocol of the CACATU study.** *BMJ Open* 2017; 7. DOI: 10.1136/bmjopen-2016-015636.

57 Riggers DS, Gurtner C, Protschka M, et al. **Intestinal S100/calgranulin expression in cats with chronic inflammatory enteropathy and intestinal lymphoma.** *Animals* 2022; 12: 2044. DOI: 10.3390/ani12162044.

58 Lingard AE, Briscoe K, Beatty JA, et al. **Low-grade alimentary lymphoma: clinicopathologic findings and response to treatment in 17 cases.** *J Feline Med Surg* 2009; 11: 692–700.