Journal of Veterinary Internal Medicine
ACVIM American College of Veterinary Internal Medicine
Open Access

Standard Article

J Vet Intern Med 2017;31:1056–1061

Evaluation of Serum 3-Bromotyrosine Concentrations in Dogs with Steroid-Responsive Diarrhea and Food-Responsive Diarrhea

P. Sattasathuchana, K. Allenspach, R. Lopes, J.S. Suchodolski, and J.M. Steiner

Background: The clinical usefulness of serum 3-BrY concentrations for subclassifying dogs with food-responsive diarrhea (FRD) and steroid-responsive diarrhea (SRD) has not been studied.

Hypothesis/Objectives: To compare serum 3-BrY concentrations in dogs with FRD, dogs with SRD, and healthy control dogs.

Animals: 38 dogs with FRD, 14 dogs with SRD, and 46 healthy dogs.

Methods: Prospective study. Measurement of 3-BrY concentration in serum samples was performed by gas chromatography/mass spectrometry.

Results: There was no association of peripheral eosinophilia in dogs with FRD, SRD, and healthy control dogs ($P = 0.069$). There was no significant correlation between peripheral eosinophil counts and serum 3-BrY concentrations ($\rho = -0.15$, $P = 0.13$). Serum 3-BrY concentrations in dogs with SRD (median [range] = 3.27, 0.9–26.23 μmol/L) were significantly higher than in dogs with FRD (median [range] = 0.99, 0.62–8.82 μmol/L; $P = 0.007$) or in healthy dogs (median [range] = 0.62, 0.62–1.79 μmol/L; $P < 0.001$). Also, serum 3-BrY concentrations in dogs with FRD were significantly higher than in healthy dogs ($P = 0.025$). There was no significant correlation between the canine chronic enteropathy clinical activity index and serum 3-BrY concentrations ($\rho = 0.17$, $P = 0.23$).

Conclusions and Clinical Importance: Measurement of serum 3-BrY concentrations, but not the peripheral eosinophil count, is helpful for detecting dogs with SRD and FRD.

Key words: Activated eosinophil; Canine; Chronic enteropathy.

Canine chronic enteropathy (CE) is a chronic inflammatory condition of unknown cause.[1–5] Clinical signs in dogs with CE may include anorexia, depression, diarrhea, vomiting, weight loss, or ascites in severe cases.[3,4] The diagnosis of CE can be achieved through treatment trials, such as dietary change, antibiotic treatment, and anti-inflammatory drug treatment. Dogs with CE that show a clinical response to a hydrolyzed protein diet or a restricted diet are classified as having food-responsive enteropathy or food-responsive diarrhea (FRD). Canine patients with CE that do not respond successfully to a hydrolyzed protein diet but show a clinical response to metronidazole or tylosin administration are classified as having antimicrobial-responsive enteropathy or antimicrobial-responsive diarrhea (ARD). Dogs with CE that have failed to respond to a hydrolyzed protein diet and also have failed to respond to metronidazole or tylosin at standard dosages, but show a clinical response to immunosuppressive drug treatment are classified as having steroid-responsive enteropathy or steroid-responsive diarrhea (SRD) or idiopathic inflammatory bowel disease.[1–5] Studies have shown that among dogs with CE, FRD is the most common type, followed by SRD and ARD.[3,4,6] Dogs with FRD have less severe clinical signs and better outcomes when compared to dogs with other types of CE.[6]

Histopathology of mucosal inflammation in the small intestine in dogs with CE has most commonly been reported as lymphocytic-plasmacytic enteritis (LPE),[7,8] with eosinophilic gastroenteritis (EGE) being the second most common form. Neutrophilic enteritis, granulomatous colitis, and histiocytic ulcerative colitis also have been reported as minor forms of histopathologic findings in dogs with CE.[7,8] An immune response to parasites or to dietary antigens is believed to be the cause of the recruitment of eosinophils into the GI tract.[9,10]

Abbreviations:

3-BrY	3-bromotyrosine
ARD	antimicrobial-responsive diarrhea
CCECAI	canine chronic enteropathy activity index
CE	chronic enteropathy
EGE	eosinophilic gastroenteritis
FRD	food-responsive diarrhea
GI	gastrointestinal
LPE	lymphocytic-plasmacytic enteritis
SRD	steroid-responsive diarrhea

From the Gastrointestinal Laboratory, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX (Sattasathuchana, Lopes, Suchodolski, Steiner); Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand (Sattasathuchana); and Department of Veterinary Clinical Sciences and Services, Royal Veterinary College, University of London, London, UK (Allenspach); Dr. Karin Allenspach's current address is Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA.

The work described in this article was performed at the Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA.

Corresponding author: P. Sattasathuchana, Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University 50 Phahonyothin Rd., Lat Yao, Jatujak, Bangkok 10900, Thailand; e-mail: psatta99@gmail.com

Submitted December 17, 2016; Revised March 31, 2017; Accepted April 19, 2017.

DOI: 10.1111/jvim.14742

The canine chronic enteropathy activity index (CCECAI) has been utilized to investigate the prognostic outcome in dogs with CE.[3] CCECAI is a combination of 9 variables including dog's attitude, appetite, vomiting, fecal consistency, fecal frequency, weight loss, albumin concentration, ascites and peripheral edema, and pruritus.[3] A high CCECAI is a negative prognostic indicator in dogs with CE.[3,4] CCECAI scores were not correlated with the histological score in 1 study.[11]

Various noninvasive tools and markers have been studied to identify eosinophil activation in the GI tract, including peripheral eosinophil counts and serum 3-bromotyrosine concentrations (3-BrY).[12–14] A durable and specific by-product of hypobromous acid, 3-BrY, is generated during a reaction catalyzed by eosinophil peroxidase (EPO) that occurs after the activation of eosinophils in the tissues.[15–17] Serum 3-BrY concentrations have been studied in both humans and canine patients with eosinophilic-related diseases.[14,18,19] There are increased serum 3-BrY concentrations in dogs with LPE and EGE, suggesting a pathophysiological role of eosinophil activation in dogs with these 2 forms of CE.[14] In the present study, we evaluated the clinical diagnostic value of peripheral eosinophil counts and serum 3-BrY concentrations in dogs with 2 forms of CE. Therefore, the objectives of this study were (1) to evaluate a possible association of peripheral eosinophilia in dogs with FRD, SRD, and healthy control dogs, (2) to determine the correlation between serum 3-BrY concentrations and peripheral eosinophil counts, (3) to compare serum 3-BrY concentrations between dogs with FRD, SRD, and healthy control dogs, and (4) to determine the relationship between CCECAI scores and serum 3-BrY concentrations.

Materials and Methods

Ethical Approvals

All serum samples of healthy control dogs were collected after approval by the Texas A&M University Institutional Animal Care and Use Committee (#2012-101), and informed consent from clients was obtained in all cases. Before enrolling healthy dogs into the study, a thorough clinical history was taken and a general physical examination was performed by veterinarian. All healthy dogs were regularly dewormed by being given anti-parasitic prevention. All clinical serum samples of dogs with FRD and SRD used for this study consisted of residual diagnostic serum samples. They were used after obtaining the written consent of the clients and were collected according to the guidelines of the Royal Veterinary College of Surgeons and the Veterinary Protection Act of 1966. In addition, the Royal Veterinary College Ethics and Welfare Committee approved (unique reference number 2013 1210) the use of these residual diagnostic serum samples for the purpose of clinical research.

Inclusion Criteria

Forty-six dogs without clinical abnormality or concurrent illness/disease were enrolled as healthy control dogs. The 52 dogs enrolled in the CE group, consisting of dogs with FRD and SRD, showed chronic diarrhea, vomiting, and/or chronic weight loss for more than 3 weeks for an unknown cause. Dogs that had clinically responded to an elimination diet or a hydrolyzed protein diet within 2 weeks after initiating dietary change and that had been kept on a strict dietary regimen for 12 weeks after diagnosis were classified as having FRD (n = 38). Dogs with ARD were defined as having failed to respond to a novel antigen of hydrolyzed dietary trial before referral that subsequently responded to metronidazole or tylosin within 2 weeks after initiation of treatment. Dogs that did not show a clinical response to dietary and antimicrobial trial with either metronidazole or tylosin before referral, and required immunosuppressive treatment to control their clinical signs, were classified as having SRD (n = 14). Dogs that were being treated with a number of combination treatments, such as prednisolone, cyclosporine, and azathioprine, were included in this group. All dogs with FRD and SRD were evaluated for CCECAI score at the time of collection. The CCECAI score for each patient was combined from 9 variables (animal's attitude, appetite, vomiting, fecal consistency, fecal frequency, weight loss, serum albumin concentration, ascites and peripheral edema, and pruritus).[3] Each variable is assessed with a score 0-3, with 0 being normal and 3 being severely abnormal. Mild, moderate, severe, and very severe clinical disease scoring corresponded to a total CCECAI score of 4-5, 6-8, 9-11, and >12, respectively.[3]

The blood sample for counting peripheral eosinophils and for the preparation of serum for the measurement of serum 3-BrY concentration was collected at the same time. The time point of blood collected was at the time of CE diagnosis and before performing any therapeutic trails. An automated hematology analyzer[a] was used to perform a CBC for each dog within 12 h after sample collection. The differential cell counts for all dogs were performed manually based on microscopic evaluation of a blood smear. Dogs with a peripheral eosinophil count of more than 1,250 cells/μL were classified as having peripheral eosinophilia, whereas dogs with peripheral eosinophil counts between 0 and 1,250 cells/μL were classified as having a normal peripheral eosinophil count.

Measurement of Serum 3-BrY Concentrations

All serum samples were stored at −80°C until analysis. Serum 3-BrY concentrations were measured by stable isotope dilution with electron ionization gas chromatography/mass spectrometry using D_3-BrY as an internal standard, as described elsewhere.[14,20]

Statistical Analyses

A normality test (Shapiro-Wilk test) was performed on all datasets. A parametric statistical method was used for evaluation of data that passed normality testing; otherwise, a nonparametric statistical method was used. The distribution of age and sex for all groups was compared by a Kruskal-Wallis test and a Pearson chi-square test, respectively.

A possible association of peripheral eosinophil counts with FRD, SRD, or being a healthy control dog was evaluated by a Fisher's exact test. The Spearman rank sum correlation was applied to determine the relationship of serum 3-BrY concentrations with peripheral eosinophil counts and CCECAI scores. The Kruskal-Wallis test also was performed to compare serum 3-BrY concentrations between dogs with FRD, dogs with SRD, and healthy control dogs. A Dunn's post-test was applied to determine differences of serum 3-BrY concentrations among groups. Statistical significance was set at a P value of <0.05. All statistic methods were performed by commercially available software package.[b,c]

Results

Among the 38 dogs with FRD, 14 dogs with SRD, and 46 healthy control dogs, there were no differences in age distribution (median ages [range]: dogs with

FRD, 2.1 years [0.5–11.1]; dogs with SRD, 5.7 years [0.6–10.7]; healthy control dogs, 4 years [1–10]; $P = 0.12$) and sex distribution (male [neutered]/female [spayed]: dogs with FRD, 14 [6]/24 [16]; dogs with SRD, 5 [4]/9 [5]; healthy control dogs, 19 [18]/27 [26]; $P = 0.89$).

Of the 38 dogs with FRD, 35 were purebred. The breeds were Boxer (5), Labrador Retriever (4), West Highland White Terrier (3), Cocker Spaniel (2), English Springer Spaniel (2), Golden Retriever (2), Rottweiler (2), Staffordshire Bull Terrier (2), American Bulldog (1), Bearded Collie (1), Border Terrier (1), Chow Chow (1), English Bull Terrier (1), German Shepherd (1), Greyhound (1), Italian Spinone (1), Miniature Dachshund (1), Miniature Poodle (1), Old English Sheepdog (1), Pointer (1), and Rhodesian Ridgeback (1). The remaining 3 dogs were of mixed breed.

The breeds of the 14 dogs with SRD were Doberman Pinscher (2), Labrador Retriever (2), Bichon Frise (1), Bull Terrier (1), Cairn Terrier (1), Hungarian Vizsla (1), Irish Setter (1), Miniature Schnauzer (1), Old English Sheepdog (1), Standard Poodle (1), Weimaraner (1), and Yorkshire Terrier (1).

Of the 46 healthy control dogs, 13 were mixed breed and 33 were purebred. The most common breeds were Australian Shepherd (6), Labrador Retriever (5), Chihuahua (3), Beagle (2), German Shepherd (2), Miniature Schnauzer (2), and Shih Tzu (2). All other breeds were represented by a single dog each: Basset Hound, Boston Terrier, Brittany Spaniel, Catahoula Hog Dog, Dachshund, English Cocker Spaniel, Poodle, Red Bone Hound, Siberian Husky, St. Bernard, and Weimaraner.

The median (range) of peripheral eosinophil counts in dogs with FRD, dogs with SRD, and healthy control dogs was 300 (0–2,280), 60 (0–1,820), and 456.5 (0–1,170) cells/μL, respectively. However, there was no association of peripheral eosinophilia in dogs with FRD, dogs with SRD, and healthy control dogs ($P = 0.069$; Fig 1). Also, there was no significant correlation between peripheral eosinophil counts and serum 3-BrY concentrations ($\rho = -0.15$, $P = 0.13$; Fig 2).

The median (range) of serum 3-BrY concentrations in dogs with FRD, SRD, and healthy control dogs was 0.99 (0.62–8.82) μmol/L, 3.27 (0.9–26.23) μmol/L, and 0.62 (0.62–1.79) μmol/L, respectively. There was a statistically significant difference in serum 3-BrY concentrations between dogs with FRD, dogs with SRD, and healthy control dogs ($P < 0.001$; Fig 3). The Dunn's post-test revealed that serum 3-BrY concentrations in dogs with SRD were significantly higher than those in dogs with FRD ($P = 0.007$) or in healthy control dogs ($P < 0.001$). In addition, serum 3-BrY concentrations in dogs with FRD were significantly higher than those in healthy control dogs ($P = 0.025$). Because clinical scoring by CCECAI was useful for assessing severity and prognostic outcome of CE,[3] the Spearman rank test was applied to identify the strength of the relationship between CCECAI score and serum 3-BrY concentrations. However, there was no significant correlation between CCECAI scores and serum 3-BrY concentrations ($\rho = 0.19$, $P = 0.23$; Fig 4).

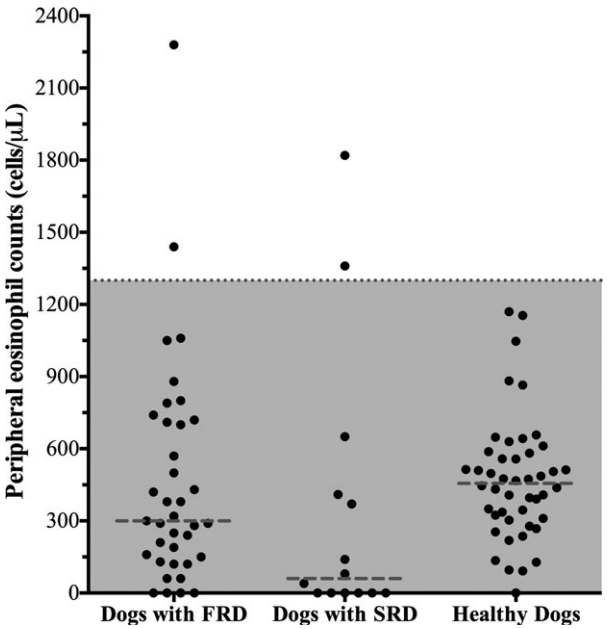

Fig 1. Scatter plot showing peripheral eosinophil counts in dogs with FRD, dogs with SRD, and healthy control dogs. There was no association between peripheral eosinophilia in dogs with FRD, dogs with SRD, and healthy dogs ($P = 0.069$). The gray area represents the reference interval for peripheral eosinophil counts in dogs (0–1,250 cells/μL). The gray dashed line shows the median of peripheral eosinophil counts for each group of dogs.

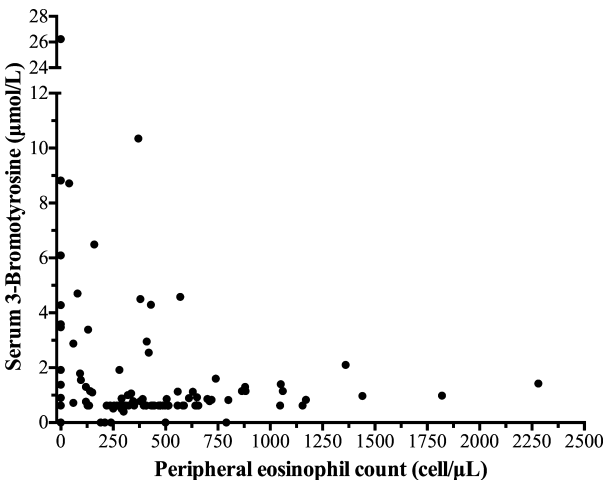

Fig 2. Scatter plot, showing the relationship between serum 3-BrY concentrations (μmol/L) and peripheral eosinophil counts (cells/μL) for all dogs enrolled in this study. The Spearman rank sum correlation test showed no significant correlation between 3-BrY concentrations and peripheral eosinophil counts ($\rho = -0.15$, $P = 0.13$).

Discussion

Diagnosing dogs with CE with no obvious cause is challenging and requires empirical treatment trials, including dietary trials, followed by antimicrobial trials, and finally antiinflammatory trials. Empirical treatment trials are time-consuming and may lead to worsening of

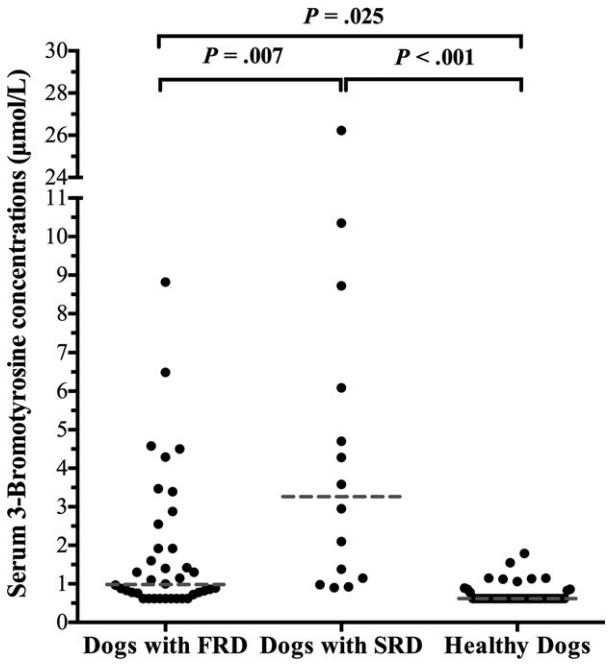

Fig 3. Scatter plot showing serum 3-BrY concentrations in dogs with FRD, dogs with SRD, and healthy control dogs. The medians for 3-BrY concentrations are shown with dashed lines. There was a statistically significant difference in serum 3-BrY concentrations between dogs with FRD, dogs with SRD, and healthy control dogs ($P < 0.001$).

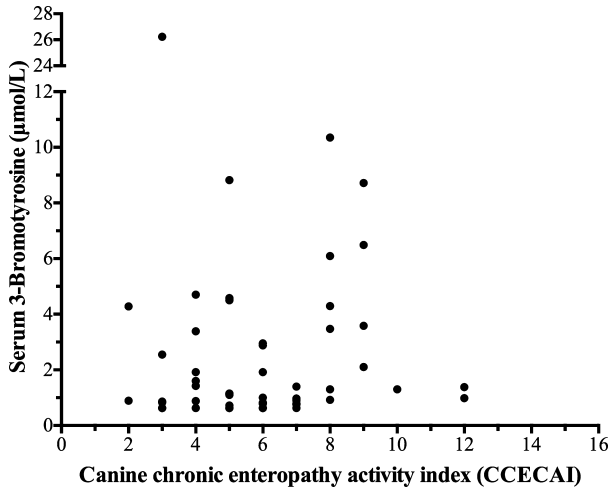

Fig 4. Scatter plot, showing the relationship between serum 3-BrY concentrations (µmol/L) and CCECAI in all dogs. The Spearman rank sum correlation test showed no significant correlation between 3-BrY concentrations and CCECAI scores ($\rho = 0.17$, $P = 0.23$).

the disease, which may necessitate invasive intestinal biopsy. Thus, the development of noninvasive biomarkers to diagnose canine CE is important for clinical practice. Furthermore, differentiating the subtypes of CE is valuable for determining an appropriate treatment trial for affected patients. This study examined 3-BrY and peripheral eosinophilia as possible biomarkers for 2 subtypes of CE: FRD and SRD.

Measuring 3-BrY concentrations in animal serum is useful in evaluating eosinophil activation in the body and monitoring disease progression. Collecting serum samples from dogs is easy and quick. Whereas peripheral eosinophil counts were not different among dogs with FRD or SRD and healthy control dogs in the current study, serum 3-BrY concentrations in dogs with either FRD or SRD were significantly higher than those in healthy control dogs. Furthermore, serum 3-BrY concentrations in dogs with SRD were higher than those in dogs with FRD. Therefore, serum 3-BrY concentrations may have clinical usefulness for diagnosing dogs with CE.

Although the peripheral eosinophil counts in healthy dogs were slightly higher than in dogs with CE, eosinophil counts were within the reference limit for all healthy control dogs (0–1,250 cell/µL). Thus, this difference in eosinophil counts between groups was not considered to be clinically important. There was no association between peripheral eosinophil counts and FRD or SRD, which is consistent with previous studies investigating dogs with CE.[14] In the present study, peripheral eosinophilia was found in 2 of the 38 dogs with FRD and 2 of the 14 dogs with SRD (Fig 1). This suggests that although peripheral eosinophil counts are easy to perform, their usefulness for differentiating dogs with SRD and FRD is relatively low.

Eosinophils only remain for a short period in circulation and predominantly dwell in the tissues.[21,22] A previous study failed to show a correlation between infiltrated eosinophils in the intestinal mucosa and eosinophil counts in the peripheral blood.[14] Because the peripheral eosinophil count does not necessarily represent the level of activation of eosinophils, the relationship between serum 3-BrY concentration and eosinophil count was evaluated by Spearman's rank correlation test (Fig 2). We were unable to identify any correlation between serum 3-BrY concentration and peripheral eosinophil count, suggesting that the clinical implication of the peripheral eosinophil count and the serum 3-BrY concentration is not synonymous.

Previous studies have compared the type of CE identified by treatment trial with histological results.[11,23,24] An increased cellularity with various inflammatory cells with either localized or diffuse infiltration of the lamina propria of the small and large intestines have been reported in dogs with both FRD and SRD.[3,11,23,25,26] Although the most commonly reported inflammatory cell type in dogs with CE are lymphocytes and plasma cells, eosinophils have also been commonly reported in dogs with CE.[3,11,23,24] In the present study, we evaluated eosinophil activation using serum 3-BrY concentrations in dogs with FRD, dogs with SRD, and healthy control dogs. The increase in 3-BrY concentrations in serum samples of dogs with FRD and SRD could suggest an activation of eosinophils. Moreover, serum 3-BrY concentrations in dogs with SRD were higher than in dogs with FRD. This may be because the severity of inflammation in dogs with SRD is higher

than in dogs with FRD, as a previous study has shown that the CCECAI in dogs with SRD was higher than in dogs with FRD.[3] In the present study, the relationship between CCECAI and serum 3-BrY concentrations was evaluated. There was no significant correlation between the CCECAI and serum 3-BrY concentrations. Both of these 2 variables were independent predictors of CE. A combination of these variables may improve the specificity for diagnosis and prognosis of CE. Further studies should be performed to evaluate the synergistic usefulness of both variables.

One limitation of this study was that dogs were classified based on clinical signs at time of presentation and clinical response to treatment along and gastrointestinal tissue biopsies were not available for these dogs. However, previous studies have shown that histopathologic findings did not always correlate with clinical signs or response to treatment in dogs with CE.[3,11,26] This may be explained by the fact that inflammatory lesions in the gastrointestinal tract can be multifocal. Therefore, the tissue sample may accidentally miss lesions during sample collection.[26] Moreover, the interpretation of histological samples may vary between pathologists.[27–29] Further studies should be performed to determine the relationship between serum 3-BrY concentrations, histopathologic findings, and clinical response to treatment in dogs with CE.

Under physiological conditions, 3-BrY is a highly specific marker for eosinophils.[30] However, 3-BrY can also be generated by neutrophils, which use chlorine to produce hypochlorous acid and chlorotyrosine, when chloride is lacking in the body or when acidosis occurs.[16] For example, in an in-vivo experiment of mice with severe sepsis, neutrophils generated 3-BrY due to a lack of chloride.[31] Fortunately, because chloride is the halide with the highest abundance in physiological fluid, a lack of chloride over bromide is unlikely to occur.[31] Another limitation of using serum 3-BrY concentrations as a marker is that serum concentrations do not allow deduction of the affected organ. Moreover, a low level of eosinophil activation may not be detected by evaluating serum 3-BrY concentrations, as a large number of samples from the healthy dogs in this study had 3-BrY concentrations below the detection limit.

Conclusions

In the present study, we evaluated the role of peripheral eosinophil counts and 3-BrY concentrations as markers for CE in dogs. We were unable to identify an association between peripheral eosinophilia and FRD or SRD in dogs. In contrast, serum 3-BrY concentrations were higher in dogs with SRD than in those with FRD or healthy control dogs. Activation of eosinophils may play a crucial role in the complex process of immune- and inflammatory response of the gut mucosa in dogs with SRD and FRD, and our findings suggest that 3-BrY may potentially serve as a noninvasive biomarker for diagnosing dogs with SRD and FRD or may be useful to evaluate progression of these diseases in dogs. Further studies are needed to further evaluate the clinical usefulness of measuring serum 3-BrY concentrations in dogs with various forms of CE.

Footnotes

[a] Cell-Dyn 3700; Abbott Diagnostics, Lake Forest, Illinois
[b] JMPPro 10, SAS Institute Inc., Cary, North Carolina
[c] GraphPad PRISM 5.0, GraphPad software, Inc., La Jolla, California

Acknowledgments

The authors acknowledge the Clinical Investigation Center (CIC) at the RVC for help with sample collection and storage.

Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Simpson KW, Jergens AE. Pitfalls and progress in the diagnosis and management of canine inflammatory bowel disease. Vet Clin North Am Small Anim Pract 2011;41:381–398.

2. Jergens AE, Moore FM, Haynes JS, et al. Idiopathic inflammatory bowel disease in dogs and cats: 84 cases (1987–1990). J Am Vet Med Assoc 1992;201:1603–1608.

3. Allenspach K, Wieland B, Grone A, et al. Chronic enteropathies in dogs: Evaluation of risk factors for negative outcome. J Vet Intern Med 2007;21:700–708.

4. Craven M, Simpson JW, Ridyard AE, et al. Canine inflammatory bowel disease: Retrospective analysis of diagnosis and outcome in 80 cases (1995–2002). J Small Anim Pract 2004;45:336–342.

5. Dandrieux JR. Inflammatory bowel disease versus chronic enteropathy in dogs: Are they one and the same? J Small Anim Pract 2016;57:589–599.

6. Allenspach K, Culverwell C, Chan D. Long-term outcome in dogs with chronic enteropathies: 203 cases. Vet Rec 2016;175:368.

7. German AJ. Small intestine. In: Washabau RJ, Day MJ, eds. Canine and Feline Gastroenterology. Saint Louis, MO: W.B. Saunders; 2013:695–699.

8. Washabau RJ. Large intestine. In: Washabau RJ, Day MJ, eds. Canine and Feline Gastroenterology. Saint Louis, MO: W.B. Saunders; 2013:729–777.

9. Kleinschmidt S, Meneses F, Nolte I, et al. Characterization of mast cell numbers and subtypes in biopsies from the gastrointestinal tract of dogs with lymphocytic-plasmacytic or eosinophilic gastroenterocolitis. Vet Immunol Immunopathol 2007;120:80–92.

10. Sattasathuchana P, Steiner JM. Canine eosinophilic gastrointestinal disorders. Anim Health Res Rev 2014;15:76–86.

11. Schreiner NM, Gaschen F, Grone A, et al. Clinical signs, histology, and CD3-positive cells before and after treatment of dogs with chronic enteropathies. J Vet Intern Med 2008;22:1079–1083.

12. Wagner M, Peterson CG, Stolt I, et al. Fecal eosinophil cationic protein as a marker of active disease and treatment outcome in collagenous colitis: A pilot study. Scand J Gastroenterol 2011;46:849–854.

13. Dainese R, Galliani EA, De Lazzari F, et al. Role of serological markers of activated eosinophils in inflammatory bowel diseases. Eur J Gastroen Hepat 2012;24:393–397.

14. Sattasathuchana P, Grutzner N, Lopes R, et al. Stability of 3-bromotyrosine in serum and serum 3-bromotyrosine concentrations in dogs with gastrointestinal diseases. BMC Vet Res 2015;11:5.

15. Weiss SJ, Test ST, Eckmann CM, et al. Brominating oxidants generated by human eosinophils. Science 1986;234:200–203.

16. Senthilmohan R, Kettle AJ. Bromination and chlorination reactions of myeloperoxidase at physiological concentrations of bromide and chloride. Arch Biochem Biophys 2006;445:235–244.

17. Mayeno AN, Curran AJ, Roberts RL, et al. Eosinophils preferentially use bromide to generate halogenating agents. J Biol Chem 1989;264:5660–5668.

18. Mita H, Higashi N, Taniguchi M, et al. Urinary 3-bromotyrosine and 3-chlorotyrosine concentrations in asthmatic patients: Lack of increase in 3-bromotyrosine concentration in urine and plasma proteins in aspirin-induced asthma after intravenous aspirin challenge. Clin Exp Allergy 2004;34:931–938.

19. Cowan DC, Taylor DR, Peterson LE, et al. Biomarker-based asthma phenotypes of corticosteroid response. J Allergy Clin Immunol 2015;135:877–883.e871.

20. Sattasathuchana P, Berghoff N, Grutzner N, et al. Development and analytic validation of an electron ionization gas chromatography/mass spectrometry (EI-GC/MS) method for the measurement of 3-bromotyrosine in canine serum. Vet Clin Pathol 2016;45:515–523.

21. Young KM, Meadows RL. Eosinophils and their disorders. In: Weiss DJ, Wardrop KJ, eds. Schalm's Veterinary Hematology. Ames, IA: Blackwell Publishing; 2010:281–289.

22. Zuo L, Rothenberg ME. Gastrointestinal eosinophilia. Immunol Allergy Clin North Am 2007;27:443–455.

23. Walker D, Knuchel-Takano A, McCutchan A, et al. A comprehensive pathological survey of duodenal biopsies from dogs with diet-responsive chronic enteropathy. J Vet Intern Med 2013;27:862–874.

24. Allenspach K, Rufenacht S, Sauter S, et al. Pharmacokinetics and clinical efficacy of cyclosporine treatment of dogs with steroid-refractory inflammatory bowel disease. J Vet Intern Med 2006;20:239–244.

25. 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.

26. Procoli F, Motskula PF, Keyte SV, et al. Comparison of histopathologic findings in duodenal and ileal endoscopic biopsies in dogs with chronic small intestinal enteropathies. J Vet Intern Med 2013;27:268–274.

27. Willard MD, Jergens AE, Duncan RB, et al. Interobserver variation among histopathologic evaluations of intestinal tissues from dogs and cats. J Am Vet Med Assoc 2002;220:1177–1182.

28. Willard MD, Mansell J, Fosgate GT, et al. Effect of sample quality on the sensitivity of endoscopic biopsy for detecting gastric and duodenal lesions in dogs and cats. J Vet Intern Med 2008;22:1084–1089.

29. Willard MD, Moore GE, Denton BD, et al. Effect of tissue processing on assessment of endoscopic intestinal biopsies in dogs and cats. J Vet Intern Med 2010;24:84–89.

30. Spalteholz H, Panasenko OM, Arnhold J. Formation of reactive halide species by myeloperoxidase and eosinophil peroxidase. Arch Biochem Biophys 2006;445:225–234.

31. Gaut JP, Yeh GC, Tran HD, et al. Neutrophils employ the myeloperoxidase system to generate antimicrobial brominating and chlorinating oxidants during sepsis. Proc Natl Acad Sci U S A 2001;98:11961–11966.