Serum calprotectin concentrations in dogs with idiopathic inflammatory bowel disease

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Objective—To measure serum calprotectin concentration in dogs with inflammatory bowel disease (IBD) before and after initiation of treatment and evaluate its correlation with a clinical scoring system (canine IBD activity index), serum canine C-reactive protein concentration, and severity of histopathologic changes.

Animals—34 dogs with idiopathic IBD and 139 healthy control dogs.

Procedures—From dogs with IBD, blood samples were collected immediately before (baseline) and 3 weeks after initiation of 1 of 2 treatments: prednisone (1 mg/kg, PO, q 12 h; n = 21) or a combination of prednisone and metronidazole (10 mg/kg, PO, q 12 h; 13). Blood samples were collected once from each of the control dogs. For all samples, serum calprotectin concentration was determined via radioimmunoassay.

Results—Mean serum calprotectin concentrations for dogs with IBD at baseline (431.1 µg/L) and 3 weeks after initiation of treatment (676.9 µg/L) were significantly higher, compared with that (219.4 µg/L) for control dogs, and were not significantly correlated with the canine IBD activity index, serum C-reactive protein concentration, or severity of histopathologic changes. The use of a serum calprotectin concentration of ≥ 296.0 µg/L as a cutoff had a sensitivity of 82.4% (95% confidence interval, 65.5% to 93.2%) and specificity of 68.4% (95% confidence interval, 59.9% to 76.0%) for distinguishing dogs with idiopathic IBD from healthy dogs.

Conclusions and Clinical Relevance—Serum calprotectin concentration may be a useful biomarker for the detection of inflammation in dogs, but the use of certain drugs (eg, glucocorticoids) appears to limit its clinical usefulness. (Am J Vet Res 2012;73:1900–1907)
ic IBD in dogs, but evidence is mounting that impaired innate immunity has a critical role in the development of idiopathic IBD. Thus, biomarkers of phagocyte activation may represent potential, clinically useful biomarkers of inflammation in dogs with idiopathic IBD. Calprotectin, the S100A8/A9 protein complex, is expressed and released into the extracellular space by activated macrophages and neutrophils and can be induced in epithelial cells. Serum concentrations of calprotectin are often increased in human patients with idiopathic IBD. Calprotectin may be involved in the expression of proinflammatory cytokines and chemokines downstream of toll-like receptor-4, which is upregulated in dogs with idiopathic IBD.

To our knowledge, serum calprotectin concentration has not been evaluated in dogs with idiopathic IBD. Therefore, the aim of the study reported here was to evaluate the serum calprotectin concentration in dogs with idiopathic IBD, compared with that in healthy dogs. For dogs with idiopathic IBD, we hypothesized that the serum calprotectin concentration would be increased prior to treatment, compared with serum calprotectin concentrations in healthy control dogs; correlated with clinical disease severity as determined by a clinical scoring system (CIBDAI); serum CRP concentration, and severity of histopathologic changes; and changed significantly in response to treatment with prednisone with or without metronidazole.

**Materials and Methods**

**Dogs with idiopathic IBD**—Between 2004 and 2006, dogs with idiopathic IBD were enrolled into a randomized controlled treatment study at the Veterinary Teaching Hospital at Iowa State University, and additional testing of serum samples acquired during that study was performed for the study reported here. The protocol for that study was approved by the Iowa State University Animal Care and Use Committee, and owner consent was obtained for each dog prior to its enrollment into the study. The inclusion criteria for dogs in that study and some of the clinicopathologic results for those dogs have been reported. Briefly, dogs were included in that study on the basis of clinical signs consistent with chronic gastrointestinal disease (ie, vomiting or diarrhea of at least 3 weeks’ duration), an inadequate response to an elimination diet for a minimum of 3 weeks in combination with treatment with metronidazole or amoxicillin-clavulanic acid for 14 days, and endoscopic and histopathologic results consistent with a diagnosis of idiopathic IBD. Also for study inclusion, dogs must not have received any anti-inflamatory, immunosuppressive, or antimicrobial medications within 14 days prior to enrollment and had to have a CIBDAI score ≥ 4. For each dog in that study, a CBC, serum biochemical analysis, urinalysis, fecal exami- nation, and abdominal radiography were performed and the concentration of serum trypsin-like immunoreactivity was determined. Additional diagnostic tests to rule out other underlying or extragastro-intestinal diseases were performed at the discretion of the attending clinician. Clinical disease activity was assessed in study dogs by the attending clinician by use of an established scoring system (CIBDAI) that evaluates the dog’s general attitude and activity, appetite, frequency of vomiting, frequency of defecation, fecal consistency, and weight loss. The overall CIBDAI score can range from 0 to 18 and distinguishes 3 categories of clinical disease severity: mild (score, 4 to 5), moderate (score, 6 to 8), and severe (score ≥ 9); a score of 0 to 3 is regarded as clinically unimportant. Of the 34 dogs with idiopathic IBD evaluated in the study reported here, 24 had a gastroduodenoscopy, 2 had a colonoscopy, and 8 had both a gastroduodenoscopy and colonoscopy performed. For biopsy specimens obtained from the intestinal tract via endoscopy, extent of inflammation was diagnosed via histopathologic criteria established by the World Small Animal Veterinary Association Gastrointestinal Standardization group and scored on a 4-point scale (0 = normal, 1 = mild, 2 = moderate, and 3 = severe histopathologic changes) by a board-certified veterinary pathologist (MRA) who was unaware of each dog’s serum calprotectin concentration.

For dogs with idiopathic IBD, a blood sample was obtained during the initial physical examination at study enrollment for determination of pretreatment measurements (baseline), after which the dogs were randomly assigned to receive either prednisone (1 mg/kg, PO, q 12 h; n = 21) or a combination of prednisone and metronidazole (10 mg/kg, PO, q 12 h; 13). Additionally, each dog’s owner was instructed to strictly adhere to feeding the dog a commercial elimination diet for 3 weeks. Dogs with hypocobalaminemia received supplemental cobalamin. Dogs that had clinical or histopathologic evidence of colitis received supplemental fiber. Each dog was reevaluated at the veterinary teaching hospital approximately 3 weeks after the initial physical examination. During this examination, another blood sample was obtained for determination of posttreatment measurements, and the dog was assigned a posttreatment CIBDAI score.

**Healthy control dogs**—Between 2004 and 2006 and 2009 and 2011, blood samples were obtained from healthy dogs (n = 139) examined at the College of Veterinary Medicine at Texas A&M University for determination of serum calprotectin concentration. Serum calprotectin concentration data for some of these dogs have been reported in another study. To be included as a healthy control dog in the study reported here, the dog could not have any condition or receive any medication known to affect the gastrointestinal tract. The study protocol was approved by the Texas A&M University Clinical Research Review Committee, and owner consent was obtained for all dogs prior to study enrollment.

**Serum calprotectin, CRP, and cobalamin concentration**—For all 34 dogs with idiopathic IBD and 139 healthy control dogs, serum calprotectin concentration was determined via an in-house radioimmunoassay developed by our laboratory group as described. Additionally, for each of 32 of 34 dogs with idiopathic IBD, serum CRP concentration was determined via a commercially available ELISA and serum cobalamin concentration was determined via a chemiluminescence assay.

**Statistical analysis**—Results were reported in accordance with the standards for reporting studies of
diagnostic accuracy. All statistical analyses were performed with commercial software. The data distributions were tested for normality via a Shapiro-Wilk W test, and the equality of variances was tested via a Brown-Forsythe test. Because the data were found to be heavily skewed to the right, the Horn algorithm was used prior to the performance of any statistical comparison; a Box-Cox transformation (\( \lambda = 0.2 \)) was applied to the data, and outliers were identified via a Tukey method that computed the 25th and 75th percentiles for the transformed data from which the interquartile range was determined (ie, 75th percentile – 25th percentile; central 50th percentile range). Lower and upper data limits were computed as follows: lower limit = 25th percentile – (3 \times interquartile range) and upper limit = 75th percentile – (3 \times interquartile range). Data with values less than the lower data limit or greater than the upper data limit were considered outliers, and analyses were performed with and without the outliers included.

For data that were normally distributed after application of the Box-Cox transformation, summary statistics were reported as means and 95% CIs that were transformed back to the original scale. A Spearman \( \rho \) was calculated for correlation analysis, an unpaired \( t \) test was used for 2-group comparisons, and a Bonferroni correction was used for multiple comparisons. For data that were not normally distributed despite the application of the Box-Cox transformation, nonparametric tests (Wilcoxon rank sum or Kruskal-Wallis) were used and the summary statistics were reported as medians and ranges.

Because our laboratory group developed the radioimmunoassay used to determine serum calprotectin concentration and a formal reference interval for dogs had not yet been established for this assay, we used the calprotectin concentration data obtained from the healthy control dogs of the present study to create a reference interval. Following identification and removal of outlier values, the data were back-transformed to the original scale and a nonparametric estimation procedure was used to calculate the reference interval on the basis of the 2.5th and 97.5th percentiles as described.

Dogs with idiopathic IBD were dichotomized as having either severely low or moderately decreased to clinically normal serum albumin (\( \leq 20 \) g/L or \( > 20 \) g/L) and cobalamin (\( \leq 200 \) ng/L or \( > 200 \) ng/L) concentrations, respectively, on the basis of cutoff values for those factors that were associated with negative prognoses. For each outcome (serum albumin or cobalamin concentration), the serum calprotectin concentration was compared between the 2 groups.

To calculate the sensitivity and specificity of serum calprotectin concentration as determined by the radioimmunoassay used in the present study for distinguishing dogs with idiopathic IBD from healthy control dogs, an ROC curve was constructed and the area under the ROC curve was calculated. The maximum likelihood ratio and Youden index were used to determine the optimal cutoff concentration of serum calprotectin for the diagnosis of idiopathic IBD.

For dogs with idiopathic IBD that had at least partial remission of clinical signs in response to treatment (ie, a posttreatment CIBDAl score that was \(< 25\%\) of the pretreatment CIBDAl score), variables representing the change from before to after treatment (posttreatment value – pretreatment value) were calculated for CIBDAl and serum CRP concentration, and the percentage change for serum calprotectin concentration was calculated as follows: (posttreatment value – pretreatment value)/pretreatment value \( \times 100\%\). Data obtained before and after initiation of treatment were analyzed via a paired \( t \) test. An ANCOVA was used to compare the effect of prednisone treatment alone with that of the prednisone and metronidazole combination treatment and to compare pretreatment serum calprotectin concentration with that after initiation of treatment. For all analyses, values of \( P \leq 0.05 \) were considered significant.

Results

Animals—Dogs with idiopathic IBD (median, 6.9 years; range, 0.5 to 16.4 years) were significantly (\( P = 0.016 \)) older than the healthy control dogs (median, 5.0 years; range, 0.6 to 13.5 years). Of the 139 healthy control dogs, 10 were sexually intact males, 64 were castrated males, 10 were sexually intact females, and 55 were spayed females. Of the 34 dogs with idiopathic IBD, 5 were sexually intact males, 6 were castrated males, 1 was a sexually intact female, and 22 were spayed females. For dogs with idiopathic IBD, the median duration of disease was 4.6 months (range, 1.0 to 18.0 months).

Serum calprotectin concentrations in healthy control dogs—The serum calprotectin concentration in healthy control dogs ranged from 28.0 to 1,123.0 \( \mu \)g/L (median, 227.3 \( \mu \)g/L; central 90th percentile, 91.2 to 528.4 \( \mu \)g/L). For 2 dogs, the serum calprotectin concentration was greater than the calculated upper data limit (ie, both were \( \geq 3.5 \times \) interquartile range). These 2 values were classified as outliers and were excluded from the calculation of the reference interval, but were retained in the analyses to determine the sensitivity, specificity, and accuracy of the radioimmunoassay. With the 2 outlier values excluded, the serum calprotectin concentrations for the remaining 137 healthy control dogs ranged from 28.0 to 695.8 \( \mu \)g/L (median, 226.7 \( \mu \)g/L; central 90th percentile, 78.0 to 543.0 \( \mu \)g/L). The calculated reference interval for serum calprotectin concentration was 76.4 to 563.6 \( \mu \)g/L.

Baseline physical examination and diagnostic findings for dogs with idiopathic IBD—None of the dogs with idiopathic IBD had evidence of dermatologic disease. Results of serum biochemical analyses performed on all 34 dogs with idiopathic IBD revealed that 10 (29.4\%) had hypoa1buminemia (serum albumin, \( < 32 \) g/L) and 9 (26.5\%) had hypoproteinemia (serum total protein, \( < 52 \) g/L). Of the 32 dogs for which serum cobalamin and CRP concentrations were determined, 7 (21.9\%) had hypocobalaminemia (serum cobalamin, \( < 251 \) ng/L) and 15 (46.9\%) had an increased CRP concentration (serum CRP, \( > 7.6 \) mg/L). The median CIBDAl score for dogs with idiopathic IBD was 6 (range, 4 to 10), and 11, 17, and 6 dogs were classified as having mild, moderate, and severe clinical disease, respec-
Endoscopic examination of the intestinal tract revealed that most dogs had mucosal lesions, which were characterized as friable, granular, erosive, or some combination of the 3. The median histopathologic grade of intestinal biopsy specimens was 1.5 (range, 1 to 3), and 17, 14, and 3 dogs were classified as having mild, moderate, and severe histopathologic changes, respectively.

Prior to treatment, mean serum calprotectin concentration was significantly ($P < 0.001$) higher for dogs with idiopathic IBD, compared with that for healthy control dogs (219.4 µg/L; 95% CI, 200.7 to 239.6 µg/L; Figure 1). Ten of 34 (29.4%) dogs with idiopathic IBD (2 of which had a serum CRP concentration within the reference interval) had a serum calprotectin concentration greater than the upper limit of the calculated reference interval, and 22 (64.7%) had a serum calprotectin concentration greater than the central 80th percentile (338.1 µg/L). On the basis of CIBDAI classification, median serum calprotectin concentration did not differ significantly ($P = 0.970$) among dogs that were classified as having mild (median, 375.2 µg/L; range, 188.5 to 1,093.0 µg/L), moderate (median, 414.0 µg/L; range, 198.9 to 789.3 µg/L), or severe (median, 429.6 µg/L; range, 240.6 to 596.2 µg/L) clinical disease, and the serum calprotectin concentration for dogs with mild to moderate (median, 408.4 µg/L; range, 188.5 to 1,093.0 µg/L) clinical disease did not differ significantly ($P = 0.892$) from that for dogs with severe clinical disease. Also, serum calprotectin concentrations did not differ significantly ($P = 0.972$) among dogs that were classified as having mild (median, 414.0 µg/L; range, 188.5 to 1,093.0 µg/L), moderate (median, 447.7 µg/L; range, 188.6 to 1,076.3 µg/L), or severe (402.7 µg/L; range, 392.0 to 515.7 µg/L) histopathologic changes on intestinal biopsy specimens, and the serum calprotectin concentration for dogs with mild histopathologic changes did not differ significantly ($P = 0.823$) from that for dogs with moderate to severe (median, 402.7 µg/L; range, 188.6 to 1,076.3 µg/L) histopathologic changes.

For dogs with severe hypoalbuminemia (serum albumin, ≤ 20 g/L; n = 5), serum calprotectin concentration ($P = 0.827$), serum CRP concentration ($P = 0.424$), CIBDAI ($P = 1.000$), and histopathologic severity ($P = 0.828$) did not significantly differ from those for dogs (29) with serum albumin concentrations > 20 g/L. Similarly, for dogs with severe hypocalcinemia (serum cobalamin, ≤ 200 ng/L; n = 4), serum calprotectin concentration ($P = 0.864$), serum CRP concentration ($P = 0.424$), CIBDAI ($P = 0.977$), and histopathologic severity ($P = 0.732$) did not significantly differ from those for dogs (28) with serum cobalamin concentrations > 200 ng/L.

Serum calprotectin concentration was positively correlated with serum CRP concentration ($P = 0.371; 95\% CI, 0.173 to 0.568$), but this correlation did not quite reach significance ($P = 0.075$). There was no correlation between serum calprotectin concentration and CIBDAI ($P = 1.000$) or histopathologic severity ($P = 1.000$). Serum CRP concentration was positively correlated with CIBDAI ($P = 0.371; 95\% CI, 0.173 to 0.568$), but this correlation did not quite reach significance ($P = 0.110$).

Clinical findings for dogs with idiopathic IBD 3 weeks after initiation of treatment—Three weeks after initiation of the assigned treatment, 23 of 34 (67.7%) dogs were in complete clinical remission, 8 (23.5%) were in partial clinical remission, and 3 (8.8%) did not respond to treatment. Thirty-one of 34 dogs (91.2%) had a CIBDAI score < 4 (ie, clinically unimportant), and 0, 2, and 1 dogs were classified as having mild, moderate, and severe clinical disease, respectively. Only 1 of 32 dogs evaluated had a serum CRP concentration that exceeded the reference interval.

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**Figure 1**—Mean diamond plot for serum calprotectin concentration in healthy control dogs (n = 139) and dogs with idiopathic IBD (34) prior to treatment with prednisone or a combination of prednisone and metronidazole. Values on the y-axis represent those for the Box-Cox transformation. Circles represent the Box-Cox transformed values for serum calprotectin concentrations for each dog. Asterisks represent the 2 outlier values that were excluded for calculation of the serum calprotectin reference interval. Each diamond represents the 95% CI, the horizontal line across the middle of each diamond represents the mean, and the horizontal lines above and below the mean line represent the overlap marks. Overlap marks were computed as the mean ± (I[2(2)]CI)/2, where I is the mean difference. For dogs with idiopathic IBD as determined by ROC analysis, this cutoff had a sensitivity of 82.4% (95% CI, 65.5% to 93.2%) and specificity of 68.4% (95% CI, 59.9% to 76.0%) for distinguishing healthy dogs from dogs with idiopathic IBD.
Serum calprotectin concentration increased for 26 of 34 (76.5%) dogs with idiopathic IBD, remained unchanged (ie, changed ≤ 15% of maximum assay variation) for 5 (14.7%), and decreased for 3 (8.8%). The mean serum calprotectin concentration (676.9 µg/L; 95% CI, 570.4 to 808.2 µg/L) for all treated dogs 3 weeks after initiation of treatment was significantly (P < 0.001) increased, compared with the mean serum calprotectin concentration (431.1 µg/L; 95% CI, 368.2 to 502.3 µg/L) for those dogs prior to treatment. Twenty-one of 34 (61.8%) dogs had serum calprotectin concentrations that exceeded the reference interval, and 33 (97.1%) dogs had serum calprotectin concentrations that exceeded the upper limit of the central 80th percentile for that of the healthy control dogs. Prior to treatment, the serum calprotectin concentration did not differ significantly (P = 0.639) between the 2 treatment groups. Three weeks after initiation of treatment, the mean serum calprotectin concentration was significantly (P = 0.006) higher for dogs that were treated with a combination of prednisone and metronidazole, compared with that for dogs that were treated with prednisone alone (Figure 2).

The change in calprotectin concentration did not differ significantly (P = 0.732) among dogs with complete remission (median, 88.2%; range, –45.3% to 824.8%), with partial remission (median, 38.8%; range, –35.9% to 304.8%), or that did not respond to treatment (median, 27.7%; range, 6.0% to 102.7%). The change in serum calprotectin concentration from before to after treatment had a weak negative correlation (ρ = –0.441; 95% CI, –0.701 to –0.077; P = 0.033) with the change in serum CRP concentration from before to after treatment. There was a positive, although not significant, correlation (ρ = 0.391; 95% CI, 0.017 to 0.669; P = 0.072) between the change in serum CRP concentration from before to after treatment and the change in CIBDAI from before to after treatment.

ROC analysis to determine optimal serum calprotectin concentration for distinguishing dogs with idiopathic IBD from healthy dogs—Serum calprotectin concentration data obtained from the healthy control dogs and dogs with idiopathic IBD of the present study were used to plot an ROC curve and determine the optimal calprotectin concentration for distinguishing dogs with idiopathic IBD from healthy dogs. The area under the ROC curve was 0.823 (95% CI, 0.751 to 0.895; P < 0.001). Maximum sensitivity (82.4%; 95% CI, 65.5% to 93.2%) and specificity (68.4%; 95% CI, 59.9% to 76.0%) were achieved when a serum calprotectin concentration of ≥ 296.0 µg/L was used as the cutoff for distinguishing dogs with idiopathic IBD from healthy dogs, with 28.9% of all dogs misclassified at this cutoff. When a serum calprotectin concentration of ≥ 240.0 µg/L was used as the cutoff, sensitivity for identifying dogs with idiopathic IBD increased to 91.2% (95% CI, 76.3% to 98.1%), but specificity for correctly identifying healthy dogs decreased to 54.0% (95% CI, 45.3% to 62.4%) and 38.7% of all dogs were misclassified.

Discussion

To our knowledge, the present study is the first to evaluate serum calprotectin concentrations in dogs with idiopathic IBD. In the present study, serum calprotectin concentration was increased in dogs with idiopathic IBD, and these results were similar to those for humans with ulcerative colitis and Crohn’s disease.13,14 An increased serum calprotectin concentration was expected as a subsequent result of the increase in the number of activated macrophages and mucosal S100-mRNA in dogs with idiopathic IBD.9,12 This suggests that calprotectin expression and secretion following activation of myelomonocytic, and possibly epithelial, cells are associated with the inflammatory response in dogs with idiopathic IBD.9

In the present study reported here, the use of a serum calprotectin concentration of ≥ 296.0 µg/L as a cutoff resulted in moderate accuracy (82.3%), sensitivity (82.4%), and specificity (68.4%) for distinguishing dogs with idiopathic IBD from healthy dogs. Although the optimal cutoff concentration for serum calprotectin in the present study differed by approximately 10-fold, the sensitivity and specificity of serum calprotectin concentration for identifying dogs with idiopathic IBD were similar to those for identifying children with idiopathic IBD.13 Although diagnostic testing and exclusion criteria used in the study reported here resulted in the exclusion of dogs with inflammation associated with diseases (eg, pancreatitis) other than IBD, mucosal expression of calprotectin was not specifically evaluated, and further research is necessary to evaluate whether inflamed intestinal mucosa is the source of increased serum calprotectin concentrations in dogs with idiopathic IBD.

In the present study, we found a positive, albeit insignificant (P = 0.075), correl-
relation between serum calprotectin and CRP concentrations in dogs with idiopathic IBD prior to treatment. An association between serum calprotectin and CRP concentrations and clinical disease activity index has been identified in children with idiopathic IBD.11 However, in another study, there was no correlation between serum calprotectin and CRP concentrations in human patients with Crohn’s disease. The fact that 2 dogs with idiopathic IBD in the study reported here had increased serum calprotectin concentrations in conjunction with serum CRP concentrations within the reference interval prior to treatment suggests that expression of these biomarkers may differ spatially or temporally. Thus, serum calprotectin concentration may be a good biomarker for the detection of inflammation in dogs that have not received certain drugs (e.g., corticosteroids) and may be useful in combination with serum CRP concentration and a clinical disease activity index to increase the diagnostic sensitivity for identification of dogs with idiopathic IBD.

The fact that serum calprotectin was not correlated with CIBDAl for the dogs of the present study is consistent with the lack of association between fecal calprotectin concentration and clinical disease activity index in humans with Crohn’s disease.21 In the study that involved humans with Crohn’s disease, fecal calprotectin concentration was significantly higher in patients with more severe disease as determined via endoscopy. In the study reported here, serum calprotectin concentration was not correlated with histopathologic disease severity. This result was similar to results of studies involving human patients with Crohn’s disease (particularly those with ileal disease) in which fecal calprotectin concentration was not associated with histopathologic disease severity, but contrasted with results of other studies involving human patients with Crohn’s disease (particularly those with ileocolonic and colonic disease) and ulcerative colitis, in which fecal calprotectin concentration was associated with histopathologic disease severity. However, in the present study, most dogs with idiopathic IBD had mild or moderate histopathologic changes, whereas only 3 dogs had severe histopathologic changes. Moreover, for the dogs of the present study, endoscopic findings were not reported in accordance with the World Small Animal Veterinary Association scoring system because those guidelines had not been introduced at the time the endoscopic examinations were performed. Therefore, an evaluation is warranted of the association between serum calprotectin concentration and histopathologic disease severity in a population of dogs with idiopathic IBD in which a higher proportion of dogs have severe histopathologic lesions and histopathologic disease severity is classified in accordance with the World Small Animal Veterinary Association scoring system.

In dogs, chronic enteropathies are diagnosed on the basis of response to treatment; an adequate clinical response to an elimination diet or antimicrobial treatment results in a diagnosis of food-responsive or antimicrobial-responsive diarrhea, respectively, whereas a clinical response only after administration of corticosteroids or other immunosuppressant drugs results in a diagnosis of idiopathic IBD. The primary objective of the present study was to evaluate serum calprotectin concentration in dogs with idiopathic IBD, compared with that in healthy dogs; the potential for serum calprotectin concentration to differentiate dogs with idiopathic IBD from dogs with food-responsive, antimicrobial-responsive, or other gastrointestinal diseases was not investigated. We assume that fecal calprotectin concentration will reflect influx or activation of inflammatory or resident cells and thus would be a more specific indicator for gastrointestinal inflammation than serum calprotectin concentration. Indeed, our laboratory group has unpublished data that indicate serum calprotectin concentration is increased in dogs with systemic inflammatory response syndrome, sepsis, and pancreatitis. Additional research is warranted to evaluate the usefulness of fecal calprotectin concentration for distinguishing dogs with idiopathic IBD from dogs with other chronic enteropathies.

Treatment with corticosteroids is considered the standard of care for dogs with idiopathic IBD, although dogs with idiopathic IBD are also often treated with metronidazole because of its antimicrobial and immunomodulatory effects. In the present study, dogs with idiopathic IBD that were treated with a combination of prednisone and metronidazole had a larger increase in serum calprotectin concentration than did dogs treated with prednisone alone, and the change in calprotectin concentration was significantly correlated with the change in serum CRP concentration. Results of a study conducted on human blood cells in vitro suggest that the increase in calprotectin concentration following corticosteroid administration may be caused by increased expression of the calprotectin complex as a result of upregulation of the immunoregulatory cytokine IL-10. In the study reported here, the fact that there was a positive, albeit not quite significant (P = 0.075), correlation between serum calprotectin and CRP concentration in dogs with idiopathic IBD prior to treatment and a negative correlation between serum calprotectin and CRP concentration 3 weeks after initiation of prednisone treatment suggests that increased calprotectin release may result from the immunomodulatory effects of glucocorticoid treatment and extracellular calprotectin may be associated with an anti-inflammatory proapoptotic effect of extracellular calprotectin. Calprotectin induces cell death by modulation of the balance between anti- and proapoptotic Bcl-2 toward the expression of proapoptotic Bcl-2 which increases in dogs with idiopathic IBD after treatment and has been suggested as a potential biomarker for treatment success. In the study reported here, the reason serum calprotectin concentration increased by a greater magnitude in dogs that were treated with a combination of prednisone and metronidazole than in dogs that were treated with prednisone alone is difficult to explain. Metronidazole inhibits neutrophil function and proinflammatory cytokine production by monocytes and macrophages but does not affect the anti-inflammatory cytokines IL-4 and IL-10. However, to our knowledge, a clinically relevant change in serum calprotectin concentration in dogs with idiopathic IBD following treatment has not been investigated. For the
dogs with idiopathic IBD in the study reported here, it is possible, although unlikely, that the increased serum calprotectin concentrations detected 3 weeks after initiation of treatment were caused by residual inflammation despite the fact that clinical remission was achieved in most of those dogs. Further research is necessary to determine the mechanism by which serum calprotectin concentration is increased in dogs with idiopathic IBD that have been successfully treated and appear to be in clinical remission.

The use of population-based reference intervals is universally accepted, but the optimal method to establish clinical reference intervals has been a matter of intense debate. Although it is generally suggested that universality accepted, but the optimal method to establish clinical reference intervals has been a matter of intense debate. 33 Although it is generally suggested that...


