Compendium

Canine Inflammatory Bowel Disease: Current and Prospective Biomarkers for Diagnosis and Management

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Abstract: *Inflammatory bowel disease (IBD) is a common gastrointestinal disorder of dogs. Current management strategies for this disease typically involve assessing the patient for resolution of clinical signs. Biologic markers that can be used to objectively assess the natural progression and predict the course of clinical disease, including response to treatment, are needed. Over the past 5 to 10 years, there has been an ongoing search for a cost-effective, minimally invasive laboratory parameter that can detect disease activity and aid in monitoring treatment. This article reviews the biomarkers currently available for evaluating dogs with IBD.*

Canine idiopathic inflammatory bowel disease (IBD) is a group of disorders characterized by persistent or recurrent clinical signs of gastrointestinal (GI) disease of undetermined cause associated with histologic evidence of inflammatory infiltration of the small and/or large intestinal mucosa.[1,2] Given that the diagnosis is histologic, the term *IBD* likely encompasses a range of disorders with as-yet undiscovered etiologies. As the etiology remains unknown, current treatment strategies are aimed at reducing or eliminating inflammation. Therefore, it would be optimal to be able to accurately assess the degree of active inflammation so that the appropriate therapy can be instituted and pursued for a proper amount of time. No current monitoring modality (clinical perception, histologic evaluation, or ultrasonography) is able to achieve this. Thus, there is a need for simple, minimally invasive or noninvasive, objective methods for evaluating intestinal inflammation, and intestinal biomarkers may fill this niche (**BOX 1**).

Box 1. Potential Monitoring Tools for Canine Inflammatory Bowel Disease

1. Clinical scoring indices
   - Clinical Inflammatory Bowel Disease Activity Index (CIBDAI)
   - Canine Chronic Enteropathy Clinical Activity Index (CCECAI)
2. Endoscopy and histopathology
3. Abdominal ultrasound
4. Serologic markers
   - C-reactive protein
   - Albumin
   - Cobalamin and folate
5. Fecal markers
   - Calprotectin
   - S100A12
   - α1-Proteinase inhibitor
   - *N*-Methylhistamine

Clinical Scoring Indices

Clinical indices remain the most widely used tools for assessing disease activity in people with IBD (e.g., Crohn disease, ulcerative colitis), either in practice or in clinical trials.[3] The most widely used index is the Crohn disease activity index, which uses eight variables to assess "global" IBD status.[4] Others have proposed a simpler Crohn scoring system that reflects subtle daily variation in GI health.[5] The Truelove and Witts definition[6] of ulcerative colitis relies on two easily measurable clinical parameters (bowel frequency and extent of macroscopic blood loss) for determination of disease activity. Still others suggest using a combination of clinical (e.g., body weight, stool consistency) and laboratory (serum albumin, erythrocyte sedimentation rate, serum acute-phase proteins) markers to more objectively quantitate intestinal inflammatory activity.[6,7]

Veterinarians rely predominantly on the severity of clinical signs to determine disease control in dogs being treated for IBD.

Table 1. Comparison of Clinical Activity Indices (CIBDAI versus CCECAI)[9,10]

	Clinical Inflammatory Bowel Disease Activity Index (CIBDAI)	Canine Chronic Enteropathy Clinical Activity Index (CCECAI)
Attitude/Activity	0 normal	0 normal
	1 slightly decreased	1 slightly decreased
	2 moderately decreased	2 moderately decreased
	3 severely decreased	3 severely decreased
Appetite	0 normal	0 normal
	1 slightly decreased	1 slightly decreased
	2 moderately decreased	2 moderately decreased
	3 severely decreased	3 severely decreased
Vomiting	0 normal	0 normal
	1 mild (1×/wk)	1 mild (1×/wk)
	2 moderate (2–3×/wk)	2 moderate (2–3×/wk)
	3 severe (>3×/wk)	3 severe (>3×/wk)
Stool consistency	0 normal	0 normal
	1 slightly soft feces	1 slightly soft feces
	2 very soft feces	2 very soft feces
	3 watery diarrhea	3 watery diarrhea
Stool frequency	0 normal	0 normal
	1 slightly increased (2–3×/d) or fecal blood, mucus, or both	1 slightly increased (2–3×/d) or fecal blood, mucus, or both
	2 moderately increased (4–5×/d)	2 moderately increased (4–5×/d)
	3 severely increased (>5×/d)	3 severely increased (>5×/d)
Weight loss	0 none	0 none
	1 mild (<5%)	1 mild (<5%)
	2 moderate (5–10%)	2 moderate (5–10%)
	3 severe (10%)	3 severe (10%)
Albumin levels		0 albumin >2.0 g/dL
		1 albumin 1.5–1.9 g/dL
		2 albumin 1.2–1.4 g/dL
		3 albumin <1.2 g/dL
Ascites and peripheral edema		0 none
		1 mild ascites or peripheral edema
		2 moderate amount of ascites/peripheral edema
		3 severe ascites/pleural effusion/peripheral edema
Pruritus		0 no pruritus
		1 occasional episodes of itching
		2 regular episodes of itching, but stops when dog is asleep
		3 dog regularly wakes up because of itching

While these signs are important, it is difficult to know if subtle improvements in clinical signs truly reflect decreased intestinal inflammation. A scoring index for disease activity in canine IBD, the clinical IBD activity index (CIBDAI), was developed and validated to help in the management of clinical patients.[8] For this disease activity score, six prominent GI signs—attitude and activity, appetite, vomiting, stool consistency, stool frequency, and weight loss—are scored from 0 to 3 based on the magnitude of their alteration from normal in a given IBD patient. The scores are added, yielding a total cumulative CIBDAI score, which is then used to

classify the disease as clinically insignificant, mild, moderate, or severe (**TABLE 1**).[9] The CIBDAI was the first attempt to develop a simple scoring system for assessment of the dynamic changes that reflect the course of IBD in dogs, and it is a useful measure of clinical signs that result from intestinal inflammation. However, the score depends primarily on subjective patient assessment, and no long-term follow-up regarding using the CIBDAI to guide anti-inflammatory therapy has been published. A second clinical scoring index, the Canine Chronic Enteropathy Clinical Activity Index (CCECAI), considers the six clinical signs included in the CIBDAI as well as albumin concentration and the presence of ascites, peripheral edema, and pruritus (**TABLE 1**).[10] These parameters were evaluated for their usefulness in predicting response to therapy as well as outcome in a prospective study of 70 dogs with chronic enteropathies.[10] Findings with this study were slightly more powerful in guiding prognosis but, again, most of the parameters were subjective. This study did predict negative outcome for dogs with chronic enteropathies.

Endoscopy and Histopathology

When used together, endoscopic evaluation of the intestinal mucosa and histologic evaluation of biopsy specimens remain the gold standard for detecting and quantifying intestinal inflammation.[11] Histologic examination is performed to distinguish normal from diseased tissue, characterize the predominant cell type of the inflammatory infiltrate, determine severity of the inflammatory infiltrate and tissue changes, and provide an accurate morphologic or etiologic diagnosis, thus facilitating the appropriate diagnosis and a rational treatment plan and prognosis. However, GI endoscopy, biopsy, and histology are costly and invasive, and repeated procedures are often impractical in a clinical setting. Additionally, a number of studies have shown that clinical improvement is not always followed by significant improvement of the histopathologic lesions.[10,12] One study showed that the total lymphocyte count in the duodenal mucosa of dogs with IBD did not change after clinically successful treatment with cyclosporine.[13] Another failed to demonstrate a strong correlation between efficacy of therapy (reflected by CIBDAI score) and severity of histologic lesions.[14] More recently, a prospective study evaluating 70 dogs with chronic enteropathies failed to show an association between the severity of histologic changes at the time of diagnosis and long-term outcome over a 3-year period.[10] Furthermore, until recently, characterization of GI inflammation has been hampered by a lack of universally accepted criteria for evaluating the histopathologic changes within a sample of mucosal tissue.[15]

With the support of the World Small Animal Veterinary Association (WSAVA), a group was convened with the purpose of developing standards for the diagnosis and treatment of GI disease in dogs and cats. One of the first tasks of this group was to develop a consensus on the normal histology of the GI tract, with the subsequent aim of developing a set of histopathologic standards for assessing the nature and severity of mucosal inflammatory and associated morphologic changes. The resulting set of guidelines, published in 2010,[16] provides a simple visual and textual description of the major inflammatory changes in the gastric body and antrum, duodenum, and colon and defines what constitutes mild, moderate, and severe pathologic change. At this time, the WSAVA scoring system has not been sufficiently evaluated with regard to whether it improves pathologist agreement. Validation with well-designed studies is still needed.

Taken together, these data suggest that there are questions concerning the value of histopathology for the assessment of dogs with IBD. However, even if histopathology has limitations in scoring disease activity in canine patients with IBD, it can be helpful in excluding other chronic enteropathies, such as neoplasia or histiocytic ulcerative colitis. Overall, a review of the evidence currently available has not identified a strong association between clinical findings and histopathologic lesions in dogs with IBD, especially when posttreatment changes in disease activity are compared with posttreatment histopathologic findings.

Abdominal Ultrasonography

Abdominal ultrasonography is an important tool to examine the GI tract of dogs with chronic vomiting and diarrhea. Intestinal wall thickness has been suggested as a criterion for determining disease activity in humans with IBD for some time.[17–19] In human IBD patients, it has been suggested that ultrasonography can be used to detect intestinal inflammation and monitor changes in disease activity during treatment.[20] Measurements of intestinal wall thickness have not been found to be specific or sensitive for the diagnosis of idiopathic IBD in dogs.[21] Also, in a separate study,[22] no significant correlation was found between the posttreatment ultrasonography score and the posttreatment CIBDAI score, despite a significantly improved CIBDAI score. Given that the ultrasonography score was associated with clinical disease activity (increased CIBDAI score) at presentation, but not after treatment, intestinal wall thickness does not appear to be helpful as a monitoring tool in dogs with inflammatory disorders. These findings likely suggest that dynamic events are occurring during the healing phase of intestinal inflammation that cannot be recognized histologically or with two-dimensional grayscale ultrasonography.

Serologic Markers

A number of serologic markers have been found to be useful in identifying and monitoring biologic disease activity in human patients with IBD. This has led to the search for minimally invasive markers of disease that can help with diagnosis, prognosis, and management of canine IBD. At this time, the number of serologic markers of IBD that have been evaluated in dogs and may show promise is limited.

C-Reactive Protein

C-reactive protein (CRP) is an integrated marker of systemic inflammation and is a member of the acute-phase reactant family of proteins in dogs. Synthesis of this group of proteins is dramatically increased during inflammatory disease, regardless of the affected organ. During inflammatory disease, hepatic synthesis of CRP is dramatically upregulated (up to a 1000-fold increase).

CRP has consistently been found to be the most useful disease activity marker for IBD in human patients.[23,24] In humans, serum CRP concentrations correlate with disease activity and histologic inflammation and are useful in predicting relapse of disease.[23,24] Additionally, it has been shown that a change in serum CRP concentration is useful for assessing the efficacy of drug therapy.[24]

A 2007 study[25] evaluated dogs with IBD and microalbuminuria. This study compared serum concentrations of tumor necrosis factor α and CRP with CIBDAI score and histopathologic grade and found that the measurement of serum CRP concentrations correlated with clinical disease activity (as assessed by the CIBDAI). This implies that severe clinical disease is accompanied by a systemic inflammatory response. Although an elevation in CRP is not specific to inflammation of the GI tract, another study by Jergens et al[8] has shown significantly increased serum CRP concentrations in dogs with moderate to severe IBD compared with healthy dogs. Because serum CRP concentration is not a disease-specific marker, the greatest clinical utility of this marker in dogs with IBD would likely be in monitoring the response to treatment. It is reasonable to expect that the institution of effective dietary or medical therapy would be associated with a decrease in serum CRP. However, further studies need to be performed to confirm these data.

Albumin

Serum albumin concentrations are routinely measured in canine patients investigated for GI disease because dogs with severe IBD often experience some protein loss through the gut mucosa. Decreased serum albumin concentration has been described as a negative prognostic indicator in two recent retrospective studies of dogs with IBD.[10,12] One study of 80 dogs reported that 12 dogs (15%) had hypoalbuminemia and an additional four dogs (5%) had panhypoproteinemia.[12] Seven of the 12 hypoalbuminemic dogs were subsequently euthanized for intractable IBD. In the other study,[10] 12 of 58 (21%) dogs with IBD initially presented with hypoalbuminemia. Of these 12 dogs, seven were panhypoproteinemic, with severe hypoalbuminemia, and three of those were eventually euthanized. Eight of 12 hypoalbuminemic dogs from this study were successfully treated with cyclosporine after failing to respond to corticosteroid treatment.[10] This suggests that early, aggressive treatment may potentially decrease mortality rates in dogs with severe IBD.

Cobalamin and Folate

Cobalamin (vitamin B_{12}) is a water-soluble vitamin of diagnostic and therapeutic importance. In companion animal medicine, most attention to cobalamin has been directed toward its use as a diagnostic marker for GI disease. In dogs, hypocobalaminemia has been predominantly described in cases of antibiotic-responsive diarrhea or exocrine pancreatic insufficiency, in which a low serum cobalamin concentration is classically seen in combination with hyperfolatemia. However, in the study by Allenspach et al that evaluated 70 dogs with various chronic enteropathies (including IBD),[10] 13 dogs were initially found to be hypocobalaminemic (<200 ng/L). The dogs with initial serum cobalamin concentrations below the cutoff value had a significantly higher chance of a negative outcome. Additionally, there was a significant association between a low serum albumin concentration and a low serum cobalamin concentration.[10]

Folate is another water-soluble B vitamin (vitamin B_9). Similar to cobalamin, changes in serum folate concentrations are more likely caused by either a decreased absorption of folate or possible alteration in the intestinal microbiota. While cobalamin can be regarded as a marker for distal small intestinal disease, folate is an indicator of proximal intestinal disease.

Perinuclear Antineutrophilic Cytoplasmic Antibodies

Perinuclear antineutrophilic cytoplasmic antibodies (pANCAs) are autoantibodies that result in a characteristic perinuclear staining pattern in granulocytes when immunofluorescence detection methods are used.[26] In human patients with IBD, pANCAs have been used as serologic markers of disease and can be found in about 50% to 80% of patients with ulcerative colitis, whereas most patients (70% to 90%) with Crohn disease are negative for pANCAs.[26,27]

Titers for pANCAs have been evaluated as diagnostic markers in canine IBD as well.[28] In one study, 31 dogs with chronic intestinal inflammation were compared with 29 dogs with acute or chronic diarrhea of known origin and with 42 healthy dogs.[27] Results indicated that pANCAs were a highly specific marker for IBD, although the sensitivity of the assay was too low to be of value as a routine screening test.[27] More recently, pANCAs were shown to be a highly specific marker versus antinuclear antibody for differentiating dogs with IBD from dogs with other GI disorders.[28] Still another study[26] has shown that pANCAs might have value as a diagnostic marker of familial protein-losing enteropathy in soft-coated wheaten terriers and may help guide treatment decisions concerning dietary management of canine food-responsive enteropathy. Taken together, these data indicate that serum pANCAs may be a sensitive biomarker for canine IBD, although the association between pANCAs and clinical disease activity has not been demonstrated.

Fecal Biomarkers

A noninvasive, simple, inexpensive, rapid, sensitive tool for the assessment of the degree and extent of intestinal inflammation would be of value in both research and clinical practice settings. Laboratory markers have the potential to serve as objective measures for the assessment of disease presence and activity. The rationale behind fecal biomarkers is that the fecal material is in direct contact with the intestinal mucosa and, therefore, should contain specific markers of mucosal disease. Such markers are likely to closely reflect the presence and degree of intestinal inflammation.

Over the past decade, several fecal parameters for assessment of disease activity in humans with IBD have been studied, while new parameters are still being developed. At this time, there are only a few fecal markers that may be of use in monitoring canine IBD.

Calprotectin

Calprotectin is a heterodimeric protein complex (S100A8/S100A9) that binds Ca^{2+} and Zn^{2+}, has antimicrobial activity, and is abundant in polymorphonuclear neutrophils (PMNs) and macrophages.[29] Calprotectin is contained in infiltrating myelomonocytic cells at sites of inflammation, where it is actively or passively released into the extracellular space as a result of cell disintegration.[29,30] Epithelial cells (e.g., keratinocytes) also express calprotectin after inflammatory activation or malignant transformation in both humans and dogs.[30] In humans, increases in serum or plasma concentrations of calprotectin have been associated with various infectious and inflammatory conditions, autoimmune disorders, and malignancies.[29,31]

Because it reflects the phagocyte turnover in vivo, calprotectin has been used as an extremely sensitive but nonspecific inflammatory marker that correlates with local and systemic signs of disease activity in humans.[29,31,32] Serum calprotectin concentrations can be used to discriminate between active and quiescent Crohn disease and are believed to be useful for monitoring clinical disease activity in humans with Crohn disease.[33] Increased fecal concentrations of calprotectin in patients with Crohn disease and ulcerative colitis have been correlated with disease activity, as determined by use of endoscopy, histologic examination, and excretion of iodine-radiolabeled PMNs.[34,35] Also, increased fecal concentrations of calprotectin in humans have been associated with GI neoplasms, infections, polyps, and the use of NSAIDs.[35,36] Measurement of fecal calprotectin concentrations is simple to perform and widely used in human medicine for diagnostic screening, for monitoring treatment response and predicting clinical relapse in patients with IBD, and for discriminating organic from nonorganic intestinal disease (such as irritable bowel syndrome).[37–39]

A radioimmunoassay for the quantification of canine calprotectin in serum and feces has been validated.[40] It has been shown that the intestinal microbiota are altered in obese mice and humans, and that obesity leads to a state of chronic subclinical intestinal inflammation. In a clinical evaluation of fecal calprotectin in obese dogs, concentrations were significantly increased only in obese research dogs that were fed ad libitum.[41]

S100A12

S100A12 is another calcium-binding protein that, similar to calprotectin, is highly abundant in neutrophils and, to a lesser extent, in macrophages and monocytes.[42] IBD in humans is commonly associated with a neutrophilic infiltrate; therefore, an increase in S100 proteins in this species is not surprising. In contrast, in dogs with IBD, inflammatory infiltrates are most often lymphocytic-plasmacytic or, less commonly, eosinophilic in nature. Thus, at initial consideration, the increase of a marker for mainly neutrophilic inflammation in dogs with IBD may be counterintuitive. However, one study has documented significantly increased mucosal S100-mRNA expression in dogs with IBD.[43] Therefore, despite the lack of an obvious neutrophilic infiltrate in dogs with IBD, an increase in S100 protein concentrations may still be expected. An assay for measurement of S100A12 in dogs was recently developed and is currently being used.[44,45]

α_1-Proteinase Inhibitor

Another fecal marker of potential interest for monitoring canine IBD is α_1-proteinase inhibitor (α_1-PI). As a result of GI disease, the integrity of the intestinal mucosa may become compromised and proteins can be lost from the interstitium into the GI lumen. α_1-PI is a plasma protein, similar in size to albumin, and is lost into the GI lumen at about the same rate as albumin and other plasma proteins, such as antithrombin III. But unlike most other plasma proteins, α_1-PI is a proteinase inhibitor and thus able to resist degradation by digestive and bacterial proteinases.[46] α_1-PI remains essentially intact in the GI lumen and can, therefore, be detected in feces by use of an immunoassay. Species-specific assays are necessary, and a canine-specific ELISA has been developed.[47] Because GI protein loss can be associated with a variety of GI and systemic disorders, the measurement of α_1-PI in feces is not specific for canine IBD. However, like calprotectin, it may have a role in monitoring disease progression and response to therapy.

Several investigators have measured fecal α_1-PI concentration in dogs with a variety of GI diseases. One study specifically evaluated whether fecal α_1-PI concentration had any correlation with serum albumin concentration (which has been shown in other studies to be associated with prognosis) and found no significant correlation between fecal α_1-PI and serum albumin concentrations in dogs with GI disease.[46] It may be interesting to evaluate whether α_1-PI is excreted early during the disease process or when disease is less severe compared with serum albumin concentration, which may only decrease when the disease is more severe. Such studies could support its use as a fair monitoring tool, particularly in dogs with IBD that are not hypoalbuminemic.

N-Methylhistamine

Histamine, a potent mediator of many physical manifestations of inflammation, is stored in granules within mast cells. Mast cells are ubiquitous in the body but exist in particularly high numbers in the skin and GI tract.[48,49] In circulation, histamine has a short half-life. Following its release from mast cells, the compound is, in part, converted to *N*-methylhistamine (NMH) via the action of histamine methyltransferase and is subsequently oxidized by monoamine oxidase.[50] NMH is considered a stable metabolite of histamine, and measurement of NMH concentration has been proposed as a method of assessing histamine release in vivo.[51] Mast cell activity and systemic release of histamine have been investigated in human patients with IBD, and disease activity reportedly correlates with urinary or fecal NMH excretion.[52,53]

An assay for measurement of NMH in canine urine and fecal samples has been developed.[54] Fecal NMH concentrations have been shown to be increased in Norwegian Lundehunds with chronic GI disease.[55] Additionally, fecal NMH concentrations were found to be elevated in sled dogs after strenuous exercise, allegedly due to histamine release secondary to mucosal mast

cell degranulation in the GI tract.[56] Currently, NMH's clinical application is still being investigated.

Conclusion

When dealing with a disease process, such as canine IBD, that has a range of clinical signs that can be cyclic in nature and often resolve spontaneously, finding a robust, repeatable, and objective scoring system of disease activity is difficult. Quantification of intestinal markers appears to be an attractive approach for providing an impartial gauge. However, the ideal parameter for the assessment of canine intestinal inflammation has yet to be established. At this time, it would appear that these markers are most useful as adjunct tools, along with clinical grading of disease severity, for assessment of therapeutic response in dogs with IBD.

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