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STANDARD ARTICLE

Evaluation of duodenal perfusion by contrast-enhanced ultrasonography in dogs with chronic inflammatory enteropathy and intestinal lymphoma

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Background: Contrast-enhanced ultrasonography (CEUS) can be used to evaluate intestinal perfusion in healthy dogs. It is helpful for diagnosing and monitoring inflammatory bowel disease in humans and could be useful for dogs with chronic intestinal diseases.

Objectives: To examine duodenal perfusion in dogs with chronic inflammatory enteropathy (CIE) and intestinal lymphoma.

Animals: Client-owned dogs with CIE (n = 26) or intestinal lymphoma (n = 7) and dogs with gastrointestinal signs but histopathologically normal duodenum (controls, n = 14).

Methods: In this cross-sectional study, dogs with CIE were classified into remission (n = 16) and symptomatic (n = 10) groups based on clinical scores determined at the time of CEUS. The duodenum was scanned after IV injection of Sonazoid® (0.01 mL/kg). CEUS-derived perfusion parameters, including time-to-peak, peak intensity (PI), area under the curve (AUC), and wash-in and wash-out rates were evaluated.

Results: The PI was significantly higher in the symptomatic CIE group (median (range); 105.4 (89.3-128.8) MPV) than in the control group (89.9 (68.5-112.2) MPV). The AUC was significantly higher in the symptomatic CIE group (4847.9 (3824.3-8462.8) MPV.sec) than in the control (3448.9 (1559.5-4736.9) MPV.sec) and remission CIE (3862.3 (2094.5-6899.0) MPV.sec) groups. The PI and clinical score were positively correlated in the CIE group. No significant differences in perfusion parameters were detected between the lymphoma and CIE groups or the lymphoma and control groups.

Conclusions and Clinical Importance: The PI and AUC can detect duodenal inflammation and hence are potentially useful for excluding a diagnosis of CIE.

KEYWORDS

CEUS, enhancement, intestinal diseases, tissue perfusion

1 | INTRODUCTION

Chronic inflammatory enteropathy (CIE) in dogs is a group of disorders characterized by persistent or recurrent gastrointestinal (GI) signs (eg, diarrhea, vomiting) without known underlying etiology.[1,2] In the diagnosis of CIE, histopathological evaluation of biopsy specimens is performed to

Abbreviations: AUC, area under the curve; CCECAI, canine chronic enteropathy clinical activity index; CEUS, contrast-enhanced ultrasonography; CIE, chronic inflammatory enteropathy; CRP, C-reactive protein; GI, gastrointestinal; IBD, inflammatory bowel disease; MPV, mean pixel value; MPV·sec, mean pixel value multiply second; PI, peak intensity; ROI, region of interest; TIC, time-intensity curve; TTP, time-to-peak; WiR, wash-in rate; WoR, wash-out rate; WSAVA, World Small Animal Veterinary Association.

confirm the presence of GI inflammation and exclude neoplasia (ie, alimentary lymphoma).[3] Unfortunately, intestinal biopsies may be delayed or never performed in dogs with debilitating conditions because of anesthetic risks associated with hypoalbuminemia. Furthermore, because current recommendations for CIE include initial dietary changes, followed by antibiotic use, and finally anti-inflammatory or immunosuppressive treatment, continuous monitoring is necessary to determine therapeutic response. Clinicians rely heavily on clinical scoring, clinicopathologic findings, and B-mode ultrasound examination to guide therapeutic decisions, but these modalities either are not GI-specific or lack correlation with therapeutic responses.[4–7] Although endoscopy with histopathological evaluation is the gold standard used to assess intestinal inflammatory activity, it is relatively invasive and cumbersome for repeated evaluations. Thus, the need remains for an alternative modality for the diagnosis and monitoring of CIE in dogs.

Contrast-enhanced ultrasonography (CEUS), with microbubbles as a contrast agent, is a noninvasive diagnostic tool that allows visualization and quantification of tissue perfusion. In human medicine, changes in the post-contrast enhancement patterns and CEUS-derived perfusion parameters of the intestine in patients with inflammatory bowel disease (IBD) have been documented and exhibit good correlation with endoscopic and histopathological features.[8,9] These findings can be attributed to microvascular reconstruction in the intestine as a direct consequence of chronic inflammation, which contributes to the pathogenesis of IBD.[10,11] Because the underlying pathogenesis of CIE in dogs shares some common features with the pathogenesis of human IBD,[12,13] we hypothesized that changes in intestinal perfusion assessed by CEUS would be useful in the diagnosis and monitoring of dogs with CIE.

Previous studies have reported that CEUS allows characterization of intestinal perfusion in healthy dogs.[14–16] The assessment of perfusion parameters derived from duodenal CEUS in healthy dogs is clinically acceptable because of its repeatability and reproducibility.[17] However, further studies in dogs with intestinal diseases are warranted to investigate the clinical applicability of this technique as a diagnostic modality. Therefore, we aimed to (i) determine the presence of changes in duodenal perfusion patterns and parameters in dogs with CIE and intestinal lymphoma compared to controls; (ii) evaluate differences in duodenal perfusion patterns and parameters between dogs with CIE and intestinal lymphoma; and (iii) examine the correlation of perfusion parameters with clinicopathologic findings, clinical scores, and histopathologic findings in dogs with CIE.

2 | MATERIALS AND METHODS

2.1 | Study population

This study employed a cross-sectional design. Dogs that presented to Hokkaido University Veterinary Teaching Hospital between September 2013 and November 2017 with either active GI signs (≥3 weeks) or a history of chronic GI signs were prospectively enrolled. Only dogs with histopathologic evaluation of the duodenum were included. Dogs with a histopathologic diagnosis of lymphoplasmacytic or eosinophilic duodenitis were included in the CIE group,[3] whereas those with an infiltration of neoplastic lymphoid cells in the duodenum were included in the intestinal lymphoma group.[18] In addition, client-owned dogs that underwent gastroduodenoscopy or laparotomy for GI signs caused by diseases other than CIE and intestinal lymphoma (eg, foreign body and gastric diseases) without histopathological lesions in the duodenum were recruited for the control group. All procedures conducted in this study were approved by the institutional animal ethical committee, and informed consent was obtained from all owners of dogs involved in this study.

2.2 | Ultrasonography

Food was withheld for a minimum of 6 hours before duodenal imaging. The duodenum was first imaged using B-mode ultrasound to assess wall thickness, layering, echogenicity, the presence of corrugation, and the presence of focal or segmental lesions.[19–22] Normal duodenal wall thickness was considered be ≤5.1 mm for dogs <20 kg, ≤5.3 mm for dogs 20-29.9 kg, and ≤6 mm for dogs >30 kg.[21] Mild thickening was defined as up to 8 mm, moderate thickening was 8-20 mm, and severe thickening was >20 mm.[19] Duodenal layering was categorized as normal (all layers identified and within normal limits), present but altered (all layers distinct, but the relative thickness of ≥1 layers was abnormal), or effaced (layers not visible).[19,23] The echogenicity of the duodenal mucosa was assessed as normal, predominantly hypoechoic, or predominantly hyperechoic.[19] The presence of hyperechoic mucosal striations also was recorded.[23]

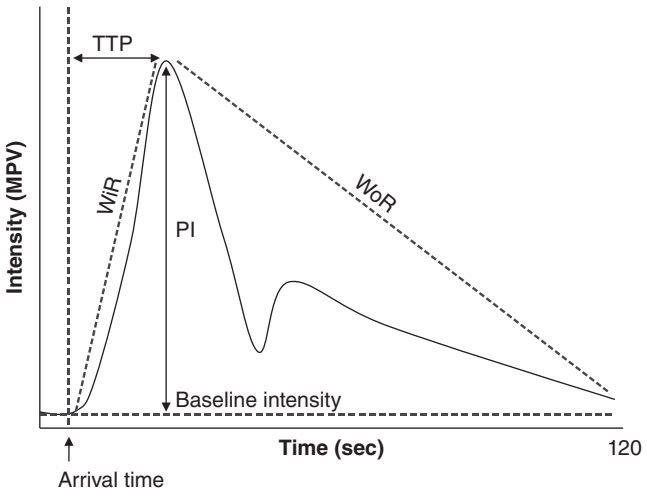

FIGURE 1 Schematic time-intensity curve (TIC) describing wash-in and wash-out after bolus injection. The arrival time indicates the time point when the intensity was greater than the baseline value followed by a continuous increase. The baseline intensity is defined as the intensity at the arrival time. The time-to-peak (TTP) indicates the duration from the first appearance of the contrast agent in the duodenal mucosa until maximum enhancement was reached. The peak intensity (PI) indicates the maximum enhancement after subtracting the baseline intensity. The area under the curve (AUC) indicates the area under the TIC above the baseline intensity calculated from the arrival time until the end of the recording (120 seconds). The wash-in and wash-out rates (WiR and WoR, respectively) were determined by performing linear regression for all values from the arrival time to the PI and from the PI to the end of the recording, respectively. MPV, mean pixel values

For CEUS scanning, dogs either were imaged with or without sedation using a combination of butorphanol (0.2 mg/kg; Vetorphale 5 mg/mL, Meiji Seika Pharma Co, Ltd, Tokyo, Japan) and midazolam (0.1 mg/kg; Dormicum 5 mg/mL, Astellas Pharma Inc, Tokyo, Japan). Contrast-enhanced ultrasonography was performed using a 5-11 MHz linear array transducer (PLT-704 AT; Aplio XG, Toshiba Medical Systems, Otawara, Japan) after IV bolus administration of a microbubble contrast agent (Sonazoid; Daiichi-Sankyo, Tokyo, Japan) at a dosage of 0.01 mL/kg. The technical parameters, including the mechanical index, image depth, focal depth, dynamic range, and gain, were consistently set at 0.20, 3 cm, 2 cm, 45 dB, and 75 dB, respectively, for all CEUS scans. The video was recorded in 40-second cine loops for a total of 120 seconds for subsequent quantitative analysis.

2.3 | Quantitative analysis

One CEUS image per second was analyzed by a single observer (K. Nisa) using image analysis software (ImageJ; US National Institutes of Health, Bethesda, Maryland). Enhancement intensity was measured by drawing 4 regions of interest (ROIs) as large as possible in the duodenal mucosa at approximately the same depth and without including major vessels or adjacent tissue. Analysis using 4 ROIs was selected because it exhibited the best repeatability compared to other methods.[17] If 4 ROIs could not be drawn because of motion artifacts, 1, 2, or 3 ROIs were drawn instead. When respiratory motion or duodenal movement was present, the ROIs were adjusted manually to maintain the same position and depth range within the duodenal mucosa. The intensity was measured at the gray scale level, with the mean pixel value (MPV) ranging from 0 to 255. The intensity means were plotted against time to create a time-intensity curve (TIC).

Five perfusion parameters were generated from the TIC, including the time-to-peak (TTP), peak intensity (PI), area under the curve (AUC), and wash-in and wash-out rates (WiR and WoR, respectively).[17] The TTP indicates the time from the first appearance of contrast agent in the duodenal mucosa until maximum enhancement is reached. The PI indicates the maximum enhancement after subtracting the baseline intensity at arrival time. The AUC indicates the area under the TIC curve above baseline intensity and is calculated from arrival time to the end of the recording. The WiR and WoR were determined by performing linear regression of all values from the arrival time to the PI and from the PI to the end of the recording, respectively (Figure 1).

TABLE 1 Signalments, clinicopathologic markers, clinical score, and histopathological score of control, chronic inflammatory enteropathy, and intestinal lymphoma groups

Variable	Control (n = 14)	Chronic inflammatory enteropathy		Intestinal lymphoma (n = 7)	Overall *P*-value*
		Remission (n = 16)	Symptomatic (n = 10)		
Signalment					
Age (years old)†	8.0 (2.0-14.0)	7.0 (5.0-12.0)	9.0 (7.0-13.0)	10.0 (7.0-12.0)	.27 (K)
Body weight (kg)†	4.0 (1.7-16.1)	4.2 (1.7-11.5)	3.9 (2.2-8.2)	5.7 (4.0-9.7)	.02 (K)
Sex	2 M, 5 CM, 7 SF	2 M, 2 F, 7 CM, 5 SF	1 M, 2 F, 4 CM, 3 SF	1 M, 4 CM, 2 SF	NE
Breed	Chihuahua (4)	Chihuahua (3)	Miniature Dachshund (5)	Miniature Dachshund (3)	NE
		Boston Terrier (2)			
		Yorkshire Terrier (2)	Boston Terrier (2)		
	Miniature Dachshund (4)	Italian Greyhound (2)	Chihuahua (1)	French Bulldog (1)	
		Japanese Spitz (1)	Welsh Corgi (1)		
		Miniature Dachshund (1)	Yorkshire Terrier (1)	Jack Russell Terrier (1)	
				Pug (1)	
	Pomeranian (2)	Miniature Schnauzer (1)		Shiba dog (1)	
	Toy Poodle (2)	Papillon (1)			
		Shih Tzu (1)			
	Miniature Pinscher (1)	Welsh Corgi (1)			
	Mix (1)				
Clinicopathologic marker					
Albumin† (RI, 2.6-4.0 g/dL)	2.6 (1.6-5.1)	2.7 (1.3-3.7)	1.7 (1.2-3.8)	1.9 (1.4-2.7)	.04 (A)
CRP† (RI, 0-1 mg/dL)	0.2 (0.0-12.0)	0.1 (0.0-1.8)	2.0 (0.0-20.0)	1.9(0.3-4.2)	.05 (K)
Clinical score					
CCECAI†,‡	NE	1.5 (0.0-3.0)[a]	6.0 (4.0-17.0)[b]	10.0 (8.0-18.0)[b]	<.0001 (K)
Histopathological score					
WSAVA†	NE	3.5 (1.0-7.0)	4.5 (1.0-7.0)	NE	.18 (T)

Abbreviations: M, Male; F, Female; CM, Castrated male; SF, Spayed female; NE, Not examined; RI, Reference interval; CRP, C-reactive protein; CCECAI, Canine Chronic Enteropathy Clinical Activity Index; WSAVA, World Small Animal Veterinary Association.
*Based on 1-way analysis of variance (A), Wilcoxon/Kruskal-Wallis (K), or student's *t* test (T).
†Values are presented as median (range).
‡Values with different superscript letters indicate significant differences among groups based on post hoc analysis (Tukey-Kramer or Steel-Dwass).

2.4 | Clinicopathologic findings, clinical scores, and histopathologic scores

Clinicopathologic findings, including plasma albumin concentrations and C-reactive protein (CRP) concentrations, were evaluated when CEUS was conducted. Furthermore, the clinical score was determined by the attending clinician based on the canine chronic enteropathy clinical activity index (CCECAI).[1] Based on the total CCECAI score, dogs in the CIE group were further classified into remission (CCECAI 0-3) and symptomatic (CCECAI >3) groups. A score of 3 was used as a cutoff value because a total CCECAI score of 0-3 is categorized as insignificant disease.[1] In addition, a single board-certified pathologist evaluated and assigned histopathological scores for the duodenum of dogs with CIE based on standards established by the World Small Animal Veterinary Association (WSAVA) GI standardization group.[3]

2.5 | Statistical analysis

The statistical analysis was performed using statistical analysis software (JMP pro 12.0.1; SAS Institute Inc, Cary, North Carolina). All data were evaluated for normality of distribution using a Shapiro-Wilk test and are presented as medians and ranges. The CEUS parameters of the control, remission CIE, symptomatic CIE, and intestinal lymphoma groups were analyzed using 1-way analysis of variance followed by a post hoc Tukey-Kramer test or a Wilcoxon/Kruskal-Wallis test followed by a post hoc Steel-Dwass test. Correlations between perfusion parameters and albumin, CRP, and CCECAI values and the WSAVA score of dogs in the CIE group were analyzed using Spearman's correlation coefficient. Statistical significance was defined as $P < .05$.

3 | RESULTS

3.1 | Study population

Thirty-three dogs were included in the CIE (n = 26) and intestinal lymphoma (n = 7) groups. Dogs in the CIE group were further classified into remission CIE (n = 16) and symptomatic CIE (n = 10) groups. Dogs in the intestinal lymphoma group were diagnosed based on histopathological evaluation of duodenal samples obtained from gastroduodenoscopy (n = 6) or laparotomy (n = 1). In addition, 14 dogs with GI signs but normal duodenal histopathological findings were recruited into the control group. The signalment, albumin concentrations, CRP concentrations, CCECAI, and WSAVA scores of all dogs are summarized in Table 1. The ages and body weights of the dogs were not significantly different among the groups. Twenty (43%) dogs were sedated before CEUS, whereas the remaining dogs (57%) underwent CEUS with manual restraint.

3.2 | B-mode ultrasound findings

The B-mode findings of the remission CIE, symptomatic CIE, and intestinal lymphoma groups are summarized in Table 2. All dogs in the remission CIE group exhibited normal wall thickness and layering. Nine dogs in the symptomatic CIE group showed normal wall thickness. One dog had mild thickening, but all dogs showed normal layering. Four dogs in the intestinal lymphoma group exhibited normal wall thickness. Three dogs exhibited normal layering, whereas 1 showed a thickened muscularis layer. The remaining 3 dogs in the intestinal lymphoma group exhibited mild thickening. One exhibited normal layering, and the other 2 dogs exhibited a thickened muscularis wall.

TABLE 2 B-mode ultrasound findings of duodenum in control, chronic inflammatory enteropathy, and intestinal lymphoma dogs

Variable	Control (n = 14)	Chronic inflammatory enteropathy		Intestinal lymphoma (n = 7)
		Remission (n = 16)	Symptomatic (n = 10)	
Wall thickness				
Normal	14	16	9	4
Mild	0	0	1	3
Moderate, severe thickening	0	0	0	0
Wall layering				
Normal	12	16	10	4
Present but altered	2	0	0	3
Effaced	0	0	0	0
Echogenicity of mucosa				
Normal	11	4	0	0
Predominantly hypoechoic	2	7	1	4
Predominantly hyperechoic	1	5	9	3
Striation	1	5	6	2
Corrugation				
Presence	2	1	1	5
Absence	12	15	9	2
Focal or segmental lesion				
Presence	0	0	0	0
Absence	14	16	10	7

Corrugation was observed in 7 dogs (remission CIE group, n = 1; symptomatic CIE group, n = 1; and intestinal lymphoma group, n = 5).

Changes in duodenal echogenicity also were observed in the majority of dogs with CIE and intestinal lymphoma. A predominantly hypoechoic duodenal mucosa was observed in 12 dogs (remission CIE group, n = 7; symptomatic CIE group, n = 1; and intestinal lymphoma group, n = 4). Predominantly hyperechoic mucosa was observed in 17 dogs (remission CIE group, n = 5; symptomatic CIE group, n = 9;

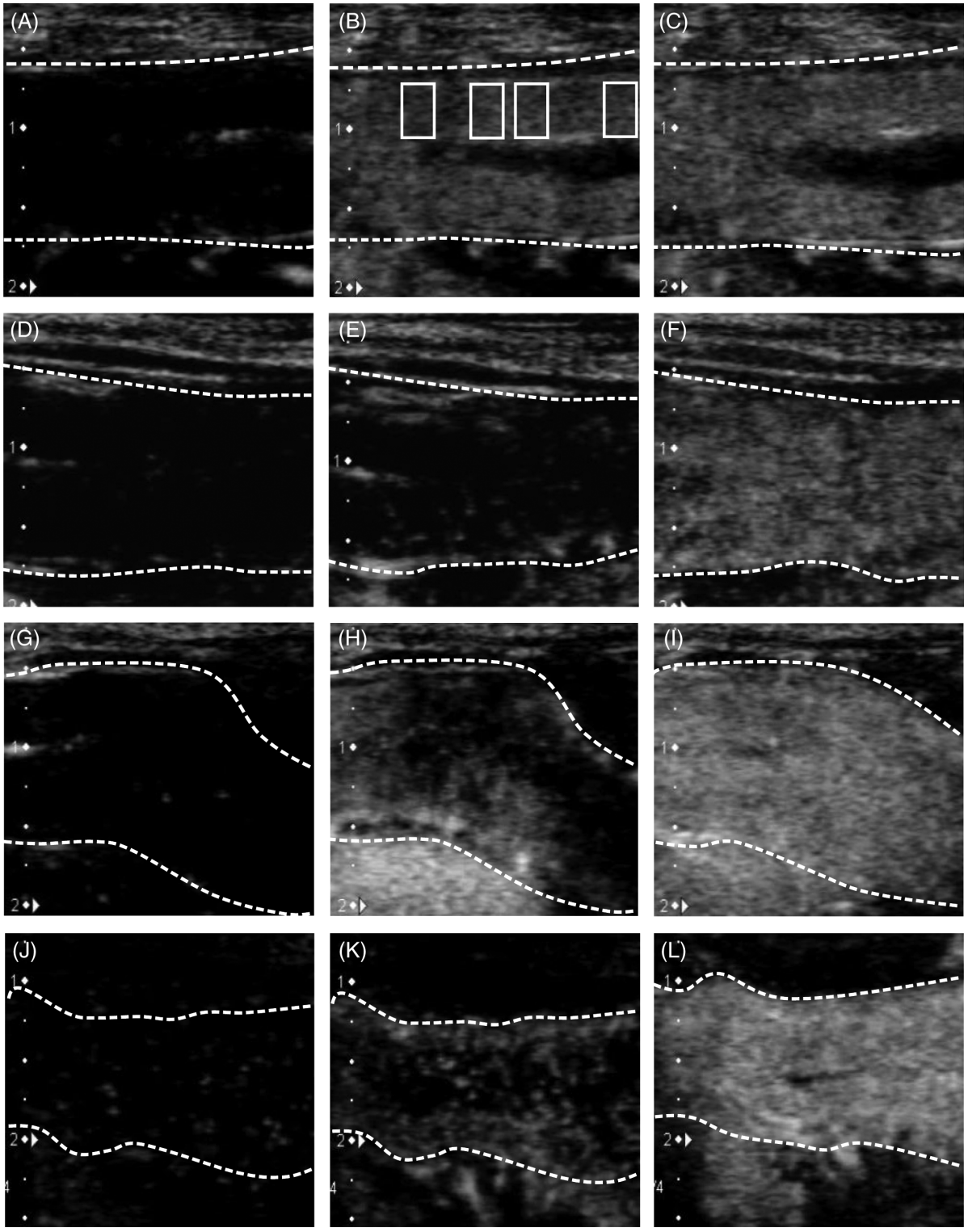

FIGURE 2 Representative sequential images of the duodenum (dashed line) after contrast injection in dogs in the control (A-C), remission chronic inflammatory enteropathy (CIE) (D-F), symptomatic CIE (G-I), and intestinal lymphoma (J-L) groups. The duodenum of representative dogs immediately before the arrival time (A, D, G, J), soon after contrast injection (B, E, H, K), and at maximum enhancement (C, F, I, L) is shown. Four regions of interests (ROIs) were drawn in the duodenal mucosa for quantitative analysis (B)

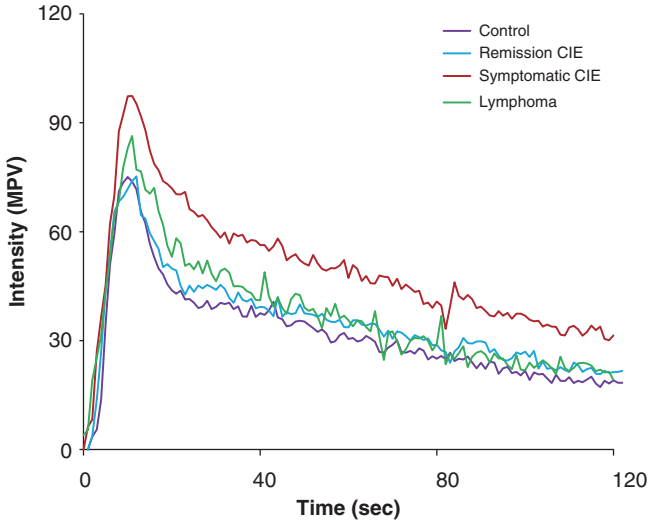

FIGURE 3 The averaged time-intensity curve (TIC) of the control (n = 14), remission chronic inflammatory enteropathy (CIE, n = 16), symptomatic CIE (n = 10), and intestinal lymphoma groups (n = 7)

intestinal lymphoma group, n = 3). Hyperechoic mucosal striations were observed in 13 dogs (remission CIE group, n = 5; symptomatic CIE group, n = 6; intestinal lymphoma group, n = 2).

3.3 | CEUS findings

The CEUS images of all dogs were adequate for analysis. The enhancement pattern of the duodenum after microbubble injection was subjectively similar between dogs in the CIE and intestinal lymphoma groups compared to the control group. Enhancement of the duodenum started from the perivisceral vessels, subsequently continued toward the mucosa (Figure 2E) and included all layers of the duodenum (Figure 2C, F,I,L). The muscularis was subjectively observed to be less enhanced than the mucosa. The enhancement of the submucosa and serosa was not included in the analysis because these layers are thin and inherently hyperechoic on ultrasound examination. Maximum enhancement in dogs in the symptomatic CIE and intestinal lymphoma groups was subjectively observed to be more prominent than that of dogs in the control and remission CIE groups (Figure 2C,F,I,L)).

In the quantitative analysis, 4 ROIs could be drawn in the duodenal mucosa of 28 dogs, 3 ROIs could be drawn in 6 dogs, 2 ROIs could be drawn in 9 dogs, and only 1 ROI could be drawn in 4 dogs. The TIC created from the average MPV of each group showed similar patterns with rapid wash-in and gradual wash-out. The TICs of the symptomatic CIE and intestinal lymphoma groups showed higher peaks than did those in the remission CIE and control groups (Figure 3). All perfusion parameters derived from the TIC are summarized in Table 3. The PI was significantly increased in the symptomatic CIE group compared to that in the control group (Table 3, Figure 4B; $P = .05$). The AUC was significantly increased in the symptomatic CIE group compared to that in the control and remission CIE groups (Table 3, Figure 4(C); $P = .009$, $P = .03$, respectively). A positive correlation was detected between the CCECAI score and the PI (Figure 5; $\rho = .55$, $P = .003$) but not with the other perfusion parameters (TTP, AUC, WiR, and WoR). No significant differences in perfusion parameters were detected between the intestinal lymphoma group and the symptomatic CIE, remission CIE, or control groups. Furthermore, no significant correlations were observed between perfusion parameters and the albumin or CRP concentrations or WSAVA score.

4 | DISCUSSION

In our study, CEUS-derived parameters (PI and AUC), which represent the regional blood volume of the duodenal mucosa, were significantly increased in dogs with symptomatic CIE compared to those in the control group. This finding suggests that the PI and AUC could be used as predictive values that suggest the presence of duodenal inflammation in dogs with active GI signs suspected of having CIE and hence could be potentially useful to exclude duodenal inflammation as a cause of the corresponding signs. However, CEUS-derived parameters were not different between dogs with CIE and intestinal lymphoma, which precludes the use of this modality to differentiate between these diseases.

Subjective observations of duodenal contrast enhancement after contrast injection showed no obvious differences in dogs in the remission CIE, symptomatic CIE, or intestinal lymphoma groups compared to the control group. The duodenal enhancement pattern observed in all dogs was consistent with the physiology of intestinal blood flow. Blood carried through small branches of splanchnic arteries initially penetrates the surface muscular coat of the duodenum, continues toward the extensive submucosal network of small arteries, and subsequently passes through the mucosal arteriole network into the microvascular bed of the mucosa.[24] Inconsistent with our results above, post-contrast enhancement patterns differ among human IBD patients

TABLE 3 Perfusion parameters of control, chronic inflammatory enteropathy, and intestinal lymphoma groups

		Chronic inflammatory enteropathy			
Variable	Control (n = 14)	Remission (n = 16)	Symptomatic (n = 10)	Intestinal lymphoma (n = 7)	Overall *P* value*
TTP (sec)†	4.0 (3.0-8.0)	4.0 (2.0-7.0)	6.0 (3.0-7.0)	6.0 (3.0-8.0)	.10 (K)
PI (MPV)†,‡	89.9 (68.5-112.2)[a]	90.9 (61.8-125.9)[ab]	105.4 (89.3-128.8)[b]	100.5 (76.7-132.4)[ab]	.04 (A)
AUC (MPV·sec)†,‡	3448.9 (1559.5-4736.9)[a]	3862.3 (2094.5-6899.0)[a]	4847.9 (3824.3-8462.8)[b]	4343.7 (2526.8-6237.0)[ab]	.01 (K)
WiR (MPV/sec)†	23.0 (10.8-31.4)	24.0 (9.3-35.5)	17.8 (13.8-47.7)	17.9 (11.7-29.4)	.23 (K)
WoR (MPV/sec)†	(–)0.7 (0.5-0.9)	(–)0.7 (0.5-0.8)	(–)0.7 (0.5-1.1)	(–)0.7 (0.6-1.0)	.53 (K)

Abbreviations: AUC, area under the curve; MPV, mean pixel value; PI, peak intensity; TTP, time to peak; WiR, wash-in rate; WoR, wash-out rate.
*Based on 1-way analysis of variance (A) or Wilcoxon/Kruskal-Wallis (K).
†Values are presented as median (range).
‡Values with different superscript letters indicate significant differences among groups based on post hoc analysis (Tukey-Kramer or Steel-Dwass).

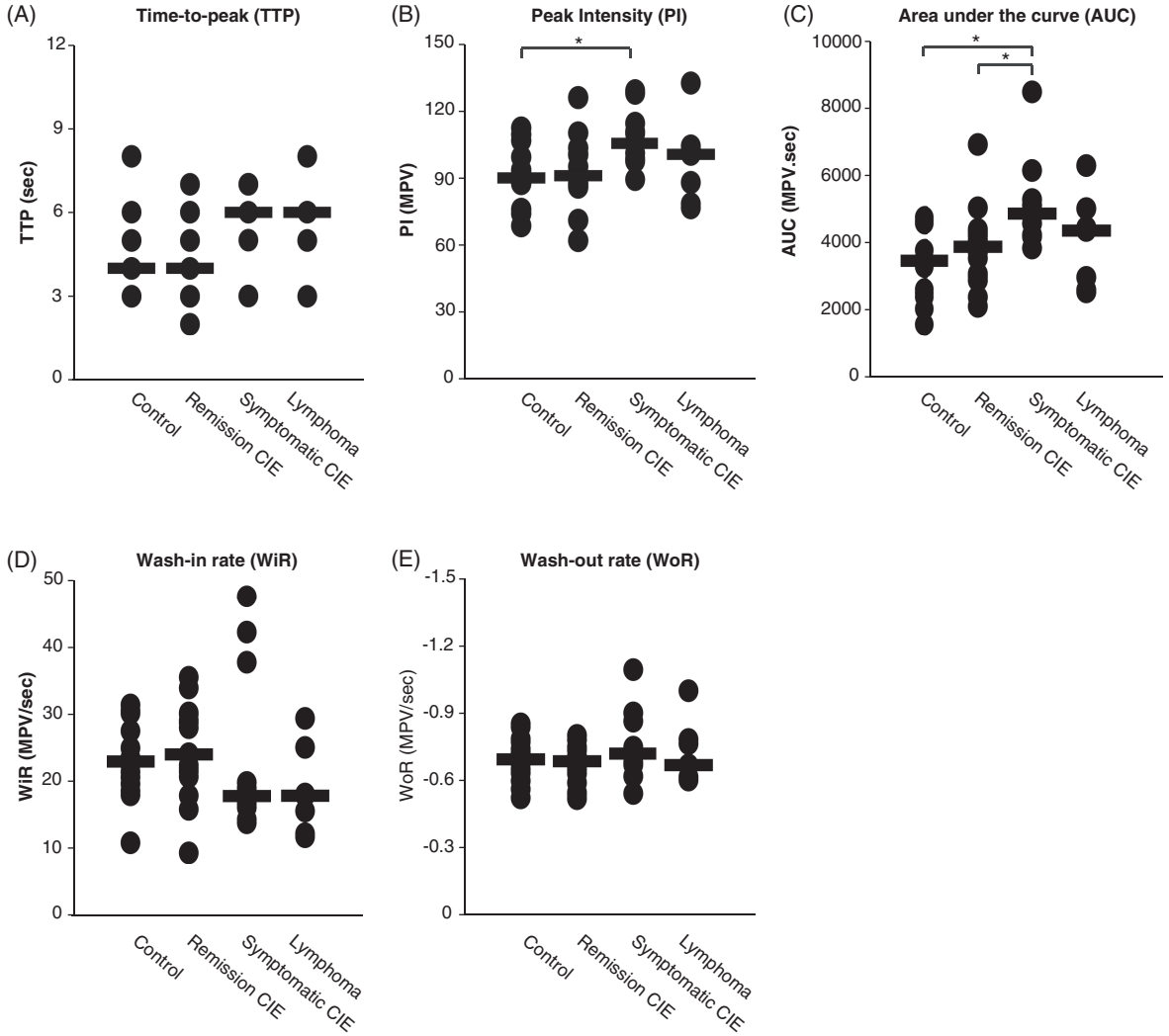

FIGURE 4 Scatter plot of perfusion parameters of the control (n = 14), remission chronic inflammatory enteropathy (Remission CIE, n = 16), symptomatic CIE (Symptomatic CIE, n = 10), and intestinal lymphoma groups (n = 7). Time-to-peak (TTP) (A), peak intensity (PI) (B), area under the curve (AUC) (C), wash-in rate (WiR) (D), wash-out rate (WoR) (E). The floating bar represents the median. An asterisk (*) indicates a significant difference among groups

with symptomatic disease and those in remission.[9] In humans with IBD, patients with symptomatic disease show prominent enhancement of the submucosa, because this layer is the most commonly affected, whereas those in remission exhibit centripetal enhancement involving all layers or low to no enhancement because of progressive fibrosis and decreased mural vascularization.[9] In addition, the distribution of the blood supply among layers in our dogs did not change. All dogs in our study exhibited less contrast enhancement in the muscularis layer than in the mucosa, because its blood supply is decreased as a result of reduced metabolic demand.[25]

Four ROIs were placed in the mucosal layer of the duodenum at the same depth for quantitative analysis. For several reasons, such as animal movement during CEUS performance or interference from the ribs, which were hard to avoid when approaching the duodenum because of the body size of small dogs, a smaller portion of the duodenum was imaged, and 4 ROIs were difficult to draw so that 3, 2, or even only 1 ROI was drawn instead. The different number of ROIs drawn might have caused variability among the samples. However, because we utilized the average MPV data of multiple ROIs for each dog, variability related to the number of ROIs should be minimized.

The PI and AUC are CEUS-derived parameters representing regional blood volume within a certain ROI. The PI represents the maximum volume of blood filling in the vessels within the ROI, whereas the AUC represents the sum of blood volume within the ROI during the period of analysis. Our findings of increased duodenal PI and AUC in dogs with symptomatic CIE correspond to an increase in blood supply with continued chronic inflammation of the duodenum. Studies in humans and mice have suggested that during chronic inflammation, vascular remodeling expands the vasculature and increases blood flow, plasma leakage, and inflammatory cell influx, which contribute to the appearance of clinical signs. Endothelial cells typically change to exhibit a venular phenotype accompanied by expression of molecules that promote endothelial gap formation and leukocyte rolling, migration, and attachment.[11,26–28]

The increase in the AUC also might be due to prolonged enhancement of the duodenal mucosa, which could have resulted from retention of microbubbles within the tortuous microvasculature of the

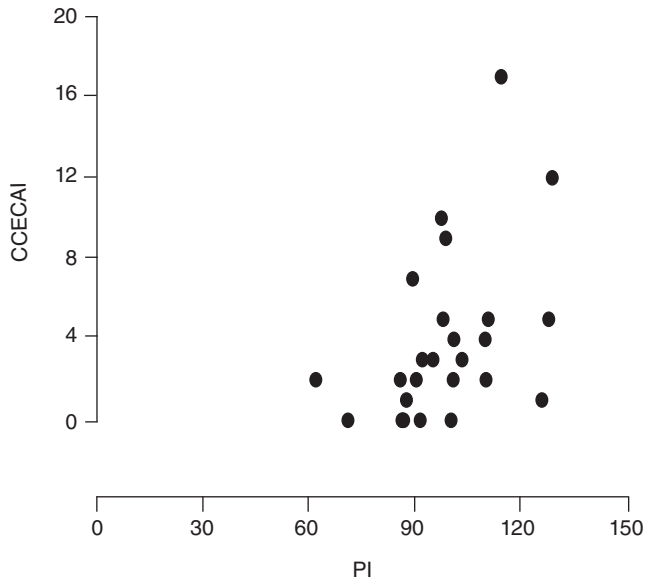

FIGURE 5 Correlation between peak intensity (PI) and the canine chronic enteropathy clinical activity index (CCECAI) in the chronic inflammatory enteropathy (CIE) group, including the remission CIE and symptomatic CIE (n = 26) groups. Spearman's rho (*r*) and the *P*-value are indicated

duodenal mucosa. In individuals with celiac disease, chronic inflammation of the intestinal mucosa causes replacement of the normal capillary architecture by a network of microvasculature with increased tortuosity and arteriovenous shunts.[29,30] The microvascular tortuosity related to chronic inflammation also was discussed in a CEUS study of dogs with pancreatitis.[31]

Although the PI and AUC of dogs in the intestinal lymphoma group were expected to be increased compared to those of the control group, no significant differences were observed in our study (Figure 4B,C). This finding could be a consequence of the small number of dogs with intestinal lymphoma in our study. An increase in the blood supply of cancerous tumors is considered to be a result of angiogenesis, which supports the survival and proliferation of cancers.[32] Several studies using contrast-enhanced computed tomography have documented various enhancement patterns of human intestinal lymphoma, with 1 study reporting inhomogeneous hyperintensity.[33–35] In addition, mild to moderate enhancement is a common feature of contrast-enhanced magnetic resonance imaging of intestinal lymphoma.[36–38] These reports suggest hypervascularization of intestinal lymphoma in human; but, this finding was not observed consistently in all cases. It is unclear whether dogs with intestinal lymphoma undergo a similar angiogenesis process, but an increase in microvascular density was reported in the lymph nodes of dogs with nodal lymphoma.[39]

We hypothesized that duodenal perfusion as assessed by CEUS would be different between dogs with CIE and lymphoma because the characteristics of vascular remodeling differ for chronic inflammation and tumors.[28] In contrast to the vascular remodeling of chronic inflamed tissue previously mentioned, tumor endothelial cells undergo disorganized sprouting, proliferation, and regression and become dependent on growth factors to survive. Thus, the new vessels increase only in number. However, the barrier function of endothelial cells is impaired, interstitial pressure and luminal resistance increased, and blood flow is decreased.[28] For these reasons, we assumed that a marked increase in duodenal perfusion indicated by an extremely high PI and AUC was more likely attributable to chronic inflammation than to neoplasia. However, our results did not identify significant differences in those parameters between the CIE and intestinal lymphoma groups. Further studies with a larger number of cases are warranted to confirm this hypothesis.

The CCECAI score was significantly correlated with the PI but not with other perfusion parameters. This correlation supports the possibility of using CEUS-derived perfusion parameters as markers for monitoring CIE in clinical practice. However, in our study, CEUS evaluation was limited to the duodenal segment. In contrast, clinicopathologic findings (eg, albumin, CRP) and the CCECAI score potentially were influenced by pathological conditions in other parts of the GI tract. This phenomenon potentially contributed to the lack of correlation between other perfusion parameters and the CCECAI. Moreover, CEUS-derived perfusion parameters did not seem to be directly correlated with the severity of morphological change and inflammatory cell infiltration in the duodenum as determined by the WSAVA scoring system.[3] Further analysis of the microvascular architecture of duodenal specimens is warranted to confirm this finding.

Our study had some limitations. The possibility that some of the control dogs suffered from CIE cannot be completely excluded because these controls were enrolled based on the presence of GI signs and the absence of histopathological abnormalities in the duodenum. In addition, histopathology of ileum was evaluated only in some dogs (data not presented). Thus inflammation or lymphoma in the ileal sections of the rest of the involved dogs might have been overlooked. Furthermore, the histopathologic findings of the ileum may not correspond to those of the duodenum.[40,41] Another limitation was the use of sedation during CEUS scanning in approximately half of our dogs. According to our previous study in healthy dogs, sedation using a combination of butorphanol and midazolam did not influence perfusion parameters of the duodenum. Because of autoregulation in the intestine, intestinal blood flow could be maintained despite the decrease in systemic blood pressure after the administration of butorphanol-midazolam.[42] Diseased dogs may respond differently to sedation than healthy dogs. However, this hypothesis cannot be confirmed because hemodynamic parameters (eg, cardiac output, heart rate, blood pressure) were not continuously recorded in our study.

In conclusion, CEUS-derived perfusion parameters, especially the PI and AUC, could indicate a change in duodenal perfusion related to chronic inflammation in dogs with CIE. These parameters could be used as predictive values that suggest the presence of duodenal inflammation in dogs with active GI signs suspected of having CIE and hence potentially could be useful to exclude duodenal inflammation as a cause of the corresponding signs. These parameters also may serve as monitoring biomarkers in dogs with CIE. Further studies with a larger number of cases and longitudinal follow-up to monitor changes in these parameters with the initiation of treatment, clinical improvement, or both are warranted to validate these assumptions.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflicts of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

This study was approved by the Animal ethical committee of Graduate School of Veterinary Medicine, Hokkaido University.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Allenspach K, Wieland B, Gröne A, Gaschen F. Chronic enteropathies in dogs: evaluation of risk factors for negative outcome. *J Vet Intern Med*. 2007;21(4):700-708.
2. Jergens AE. Clinical assessment of disease activity for canine inflammatory bowel disease. *J Am Anim Hosp Assoc*. 2004;40(6):437-445.
3. 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(1):10-26.
4. Mapletoft EK, Allenspach K, Lamb CR. How useful is abdominal ultrasonography in dogs with diarrhoea? *J Small Anim Pract*. 2018;59(1):32-37.
5. Gaschen L, Kircher P, Stüssi A, et al. Comparison of ultrasonographic findings with clinical activity index (CIBDAI) and diagnosis in dogs with chronic enteropathies. *Vet Radiol Ultrasound*. 2008;49(1):56-64.
6. Collins MT. Canine inflammatory bowel disease: current and prospective biomarkers for diagnosis and management. *Compend Contin Educ Vet*. 2013;35(3):E5.
7. Rudorf H, van Schaik G, O'Brien RT, Brown PJ, Barr FJ, Hall EJ. Ultrasonographic evaluation of the thickness of the small intestinal wall in dogs with inflammatory bowel disease. *J Small Anim Pract*. 2005;46(7):322-326.
8. Quaia E. Contrast-enhanced ultrasound of the small bowel in Crohn's disease. *Abdom Imaging*. 2013;38(5):1005-1013.
9. Migaleddu V, Scanu AM, Quaia E, et al. Contrast-enhanced ultrasonographic evaluation of inflammatory activity in Crohn's disease. *Gastroenterology*. 2009;137(1):43-61.
10. Alkim C, Alkim H, Koksal AR, et al. Angiogenesis in inflammatory bowel disease. *Int J Inflam*. 2015;2015:970890.
11. Danese S, Sans M, de la Motte C, et al. Angiogenesis as a novel component of inflammatory bowel disease pathogenesis. *Gastroenterology*. 2006;130(7):2060-2073.
12. Simpson KW, Jergens AE. Pitfalls and progress in the diagnosis and management of canine inflammatory bowel disease. *Vet Clin Small Anim*. 2011;41(2):381-398.
13. Cerquetella M, Spaterna A, Laus F, et al. Inflammatory bowel disease in the dog: differences and similarities with humans. *World J Gastroenterol*. 2010;16(9):1050-1056.
14. Jiménez DA, O'Brien RT, Wallace JD, Klocke E. Intraoperative contrast-enhanced ultrasonography of normal canine jejunum. *Vet Radiol Ultrasound*. 2011;52(2):196-200.
15. Johnson-Neitman JL, O'Brien RT, Wallace JD. Quantitative perfusion analysis of the pancreas and duodenum in healthy dogs by use of contrast-enhanced ultrasonography. *Am J Vet Res*. 2012;73(3):385-392.
16. Lim SY, Nakamura K, Morishita K, et al. Qualitative and quantitative contrast enhanced ultrasonography of the pancreas using bolus injection and continuous infusion methods in normal dogs. *J Vet Med Sci*. 2013;75(12):1601-1607.
17. Nisa K, Lim SY, Shinohara M, et al. Repeatability and reproducibility of quantitative contrast-enhanced ultrasonography for assessing duodenal perfusion in healthy dogs. *J Vet Med Sci*. 2017;79(9):1585-1590.
18. Frank JD, Reimer SB. Clinical outcomes of 30 cases (1997-2004) of canine gastrointestinal lymphoma. *J Am Anim Hosp Assoc*. 2007;43(6):313-321.
19. Frances M, Lane AE, Lenard ZM. Sonographic features of gastrointestinal lymphoma in 15 dogs. *J Small Anim Pract*. 2013;54(9):468-474.
20. Penninck DG, Smyers B, Webster CRL, Rand W, Moore AS. Diagnostic value of ultrasonography in differentiating enteritis from intestinal neoplasia in dogs. *Vet Radiol Ultrasound*. 2003;44(5):570-575.
21. Delaney F, O'Brien RT, Waller K. Ultrasound evaluation of small bowel thickness compared to weight in normal dogs. *Vet Radiol Ultrasound*. 2003;44(5):577-580.
22. Moon ML, Biller DS, Armbrust LJ. Ultrasonographic appearance and etiology of corrugated small intestine. *Vet Radiol Ultrasound*. 2003;44(2):199-203.
23. Sutherland-Smith J, Penninck DG, Keating JH, Webster CRL. Ultrasonographic intestinal hyperechoic mucosal striations in dogs are associated with lacteal dilation. *Vet Radiol Ultrasound*. 2007;48(1):51-57.
24. Washabau RJ. Integration of gastrointestinal function. In: Washabau RJ, Day MJ, eds. *Canine & Feline Gastroenterology*. St. Louis, MO: Saunders; 2013:1-31.
25. Granger DN, Richardson PDI, Kvietys PR, Mortillaro NA. Intestinal blood flow. *Gastroenterology*. 1980;78(4):837-863.
26. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med*. 2000;6(4):389-395.
27. Majno G. Chronic inflammation: links with angiogenesis and wound healing. *Am J Pathol*. 1998;153(4):1035-1039.
28. McDonald DM. Angiogenesis and vascular remodeling in inflammation and cancer: biology and architecture of the vasculature. In: Figg W, Folkman J, eds. *Angiogenesis*. Boston, MA: Springer; 2008:17-33.
29. Gustafon T, Sjolund K, Berg N. Intestinal circulation in coeliac disease: an angiographic study. *Scand J Gastroenterol*. 1982;17(7):881-885.
30. Masselli G, Picarelli A, Di Tola M, et al. Celiac Disease: evaluation with dynamic contrast-enhanced MR imaging. *Radiology*. 2010;256(3):783-790.
31. Lim SY, Nakamura K, Morishita K, et al. Quantitative contrast-enhanced ultrasonographic assessment of naturally occurring pancreatitis in dogs. *J Vet Intern Med*. 2015;29(1):71-78.
32. Ichihara E, Kiura K, Tanimoto M. Targeting angiogenesis in cancer therapy. *Acta Med Okayama*. 2011;65(6):353-362.
33. Fernandes T, Oliveira MI, Castro R, Araújo B, Viamonte B, Cunha R. Bowel wall thickening at CT: simplifying the diagnosis. *Insights Imaging*. 2014;5(2):195-208.
34. Lo Re G, Federica V, Midiri F, et al. Radiological features of gastrointestinal lymphoma. *Gastroenterol Res Pract*. 2016;2016:2498143.
35. Macari M, Balthazar EJ. CT of bowel wall thickening: significance and pitfalls of interpretation. *Am J Roentgenol*. 2001;176(5):1105-1116.
36. Crusco F, Pugliese F, Maselli A, et al. Malignant small-bowel neoplasms: spectrum of disease on MR imaging. *Radiol Med*. 2010;115(8):1279-1291.
37. Masselli G, Casciani E, Polettini E, Laghi F, Gualdi G. Magnetic resonance imaging of small bowel neoplasms. *Cancer Imaging*. 2013;13(1):92-99.
38. Semelka RC, John G, Kelekis NL, Burdeny DA, Ascher SM. Small bowel neoplastic disease: demonstration by MRI. *J Magn Reson Imaging*. 1996;6(6):855-860.
39. Woldemeskel M, Mann E, Whittington L. Tumor microvessel density–associated mast cells in canine nodal lymphoma. *SAGE Open Med*. 2014;2:2050312114559575.
40. Procoli F, Mõtsküla PF, Keyte SV, Priestnall S, Allenspach K. Comparison of histopathologic findings in duodenal and ileal endoscopic

biopsies in dogs with chronic small intestinal enteropathies. *J Vet Intern Med*. 2013;27(2):268-274.

41. Casamian-Sorrosal D, Willard MD, Murray JK, Hall EJ, Taylor SS, Day MJ. Comparison of histopathologic findings in biopsies from the duodenum and ileum of dogs with enteropathy. *J Vet Intern Med*. 2010;24:80-83.
42. Nisa K, Lim SY, Osuga T, et al. The effect of sedation with a combination of butorphanol and midazolam on quantitative contrast-enhanced ultrasonography of duodenum in healthy dogs. *J Vet Med Sci*. 2018;80(3):453-459.

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