

Contrast-enhanced ultrasonography in dogs with inflammatory bowel disease

Nikolina Linta | Pascaline Pey  | Marco Baron Toaldo  | Marco Pietra  |
 Manuel Felici | Giuliano Bettini  | Mario Cipone | Alessia Diana 

Department of Veterinary Medical Science,
 Alma Mater Studiorum, University of Bologna,
 Ozzano Emilia, Italy

Correspondence

Pascaline Pey, Department of Veterinary
 Medical Science, Alma Mater Studiorum,
 University of Bologna, Ozzano Emilia (BO),
 Italy.
 Email: pascaline.pey@unibo.it

Present address

Pascaline Pey, Ospedale Veterinario I portoni
 Rossi, Bologna (BO), Italy and Antech Imaging
 Services, Irvine, California

Marco Baron Toaldo, Universität Zürich -
 Vetsuisse-Fakultät, Klinik für Kleintiermedizin,
 Winterthurerstrasse 260, Zürich, Switzerland

Abstract

Background: Contrast-enhanced ultrasonography (CEUS) is used to evaluate vascularity of the gastrointestinal wall in neoplastic and inflammatory diseases.

Objective: To assess the feasibility of CEUS for the evaluation of duodenal perfusion in dogs with inflammatory bowel disease (IBD).

Animals: Forty-two dogs with IBD and 20 clinically healthy dogs.

Methods: All CEUS studies of the duodenum were analyzed to obtain time-intensity curves and perfusion parameters. The procedure was repeated in 12 IBD dogs 2 months after a standardized treatment.

Results: On CEUS, the duodenal wall showed a typical perfusion pattern characterized by a radial and simultaneous enhancement of the wall in all dogs. On qualitative assessment, no differences were observed in contrast medium distribution between healthy and affected dogs, or between dogs with IBD before and after treatment. Peak intensity (PI) and area under the curve (AUC) significantly differed between healthy (PI = 3.58 arbitrary units [au; 1.86-4.93 au] and AUC = 47.63 au seconds [aus, 22.68-62.15]) and affected dogs (PI = 5.10 au [0.63-15.16 au] and AUC = 63.62 aus [5.31-212.20 aus]; $P = .03$ and $.03$, respectively). No significant differences were found for the perfusion parameters before and after treatment.

Conclusions and Clinical Importance: We showed that CEUS allows discrimination between IBD affected dogs and healthy dogs by evaluation of time-intensity curves, but did not provide useful information for monitoring therapeutic response. The qualitative assessment identified no significant differences between healthy and affected dogs, or between dogs before and after treatment.

KEYWORDS

canine, gastroenterology, imaging, intestine, ultrasound

Abbreviations: 95% CI, 95% confidence interval; AT, arrival time; AUC, area under the curve; CCECAL, canine chronic enteropathy activity index; CEUS, contrast-enhanced ultrasonography; CRP, C-reactive protein; GI, gastrointestinal; IBD, inflammatory bowel disease; ICC, intraclass correlation coefficient; MTT, mean transit time; PI, peak intensity; ROC, receiver operating characteristic; ROI, region of interest; Se, sensitivity; Sp, specificity; TTPin, time to peak from initial rise; TTPj, time to peak from injection; US, ultrasound; Wi, wash-in rate.

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1 | INTRODUCTION

Inflammatory bowel disease (IBD) is a general term for a group of chronic enteropathies characterized by persistent or recurrent gastrointestinal (GI) signs, intestinal wall inflammatory infiltrates, and response to treatment using antigen-restricted diets, antimicrobials, and immunosuppressive drugs.^{1,2} The diagnosis of IBD in dogs requires careful integration of signalment, history, physical findings, laboratory test results, diagnostic imaging, histopathology of intestinal biopsy specimens, and response to treatment.³ The canine IBD activity index (CIBDAI) and the canine chronic enteropathy activity index (CCECAI) were proposed to assess disease activity, response to treatment, and long-term prognosis.^{1,3} Abdominal ultrasound (US) examination can be useful for evaluation of intestinal wall thickness and layering, as well as to identify focal or diffuse intestinal wall abnormalities and involvement of mesenteric lymph nodes.^{4,5} However, overlap between the sonographic appearances of intestinal inflammatory and neoplastic infiltration can occur, and a definitive ultrasonographic diagnosis remains difficult.⁶

In humans, contrast-enhanced ultrasonography (CEUS) has been used to evaluate the vascularity of the GI wall in neoplastic⁷ and inflammatory diseases.⁸ In particular, increased bowel vessel density, detected using a second-generation US contrast agent, is correlated with disease activity in patients with Crohn's disease.^{8,9} Treatment response in patients with Crohn's disease and ulcerative colitis using CEUS quantification also has been studied.¹⁰⁻¹²

In veterinary literature, information regarding the use of CEUS is limited to reports that evaluate normal bowel perfusion in dogs¹³⁻¹⁶ and cats,^{17,18} and as a case series that describes the use of CEUS in the diagnosis of intestinal ischemia in cats.¹⁹ An abstract has described the use of CEUS as a noninvasive imaging method for the diagnosis of IBD²⁰ and another study described the use of CEUS for the assessment of bowel perfusion in dogs with chronic inflammatory enteropathy and intestinal lymphoma.²¹

The first aim of our study was to prospectively assess the feasibility of CEUS in the evaluation of duodenal perfusion in a population of dogs with IBD and to describe the enhancement pattern. The second aim was to assess the utility of CEUS in the follow-up of dogs that received standardized treatment.

2 | MATERIALS AND METHODS

2.1 | Study design

This single-center, prospective observational study was divided into 2 parts. In the first part, the CEUS examination of the duodenum was performed in clinically healthy dogs (control group) and in dogs with IBD, and a qualitative and quantitative assessment of intestinal perfusion was compared between the groups. During the second part of the study, the CEUS examination was repeated in 12 dogs with IBD that received standardized treatment.

Written informed consent was obtained from all owners and the protocols of this study were approved by the Ethical Committee of the University Teaching Hospital (No. 28-IX/9).

2.2 | Animals

Dogs presented to the Veterinary Teaching Hospital of our institution between November 2014 and January 2016 with either current GI signs (for at least 6 weeks) or a history of chronic GI signs were prospectively enrolled (IBD group). Only dogs with clinical and histopathologic diagnosis of duodenal IBD were included in the study, whereas dogs with other disorders causing GI signs (eg, non-GI disorders, bacterial and parasitic infections, exocrine pancreatic insufficiency, or anatomical GI disorders needing surgical intervention) and those dogs already receiving drug treatment or vitamin supplementation were excluded.

Healthy dogs owned by students and hospital staff also were recruited (control group). Dogs of the control group were considered healthy on the basis of normal results of physical examination (including absence of GI signs for at least 3 weeks before starting the study), CBC, serum biochemistry profile, fecal flotation, and abdominal US examination.

All of the procedures described below were performed in healthy dogs (control group) and in all dogs with IBD (IBD group) at the time of diagnosis (T0). For 12 of these dogs (treatment group), the procedures also were repeated 62 to 68 days (T1) after the start of standardized treatment. All dogs received a hypoallergenic diet that contained a protein source to which the dogs had no prior exposure, tylosin at a dosage of 10 mg/kg PO q12h and prednisolone at a dosage of 1 mg/kg PO q24h.

2.3 | Clinicopathological findings, clinical score, endoscopic score, and histologic score

A CBC, serum biochemistry profile, canine trypsin-like immunoreactivity, and serum folate, cobalamin (B12), and C-reactive protein (CRP) concentrations, and fecal flotation were performed in all dogs with IBD at inclusion.

The severity of clinical disease (activity) for IBD dogs at the time of diagnosis was scored using CCECAI as previously described.²² Based on CCECAI results, dogs in the IBD group were classified into 5 subgroups: 0 to 3, minimal disease; 4 to 5, mild disease; 6 to 8, moderate disease; 9 to 11, severe disease; and ≥ 12 , very severe disease.²² For statistical comparison, these 5 subgroups were decreased to 4 by incorporating severe and very severe animals into a single subgroup.

Gastroenteroscopy was performed by using a video-endoscope (Pentax EG-2931, Pentax Italia, Milano, Italy). Endoscopic videos of the duodenum were recorded and an endoscopic score was assigned following previously described criteria.³ Briefly, the endoscopic appearance of the duodenum was evaluated for the following criteria: granularity, friability, erosion, and lymphatic dilatation. For each of

these variables a value (0-2) was assigned based on the presence and extent of abnormal mucosal appearance.³

In addition, the duodenal biopsy specimens were processed and examined, and a histological score was assigned according to the World Small Animal Veterinary Association's standardized guidelines.²³ Mucosal changes were defined by morphological abnormalities (villous stunting, epithelial injury, crypt distension, lacteal dilatation, and mucosal fibrosis) and the major types of inflammatory cell infiltrating the epithelium and lamina propria (intraepithelial lymphocytes, lamina propria lymphocytes and plasma cells, lamina propria eosinophils, and lamina propria neutrophils). The simple numerical addition of grades of histopathological change (normal = 0, mild = 1, moderate = 2, and marked = 3) provided an overall histological score for the tissue of interest.²³

2.4 | Ultrasound procedures and analysis

A complete abdominal ultrasonographic examination associated with CEUS of the duodenum was performed in all dogs (IBD group and control group) using only manual restraint. The dogs were fasted overnight (at least 12 hours) before imaging. All ultrasonographic procedures were conducted by the same sonographer, using a real-time ultrasound machine (iU22 ultrasound system, Philips Healthcare, Amsterdam, the Netherlands). For US scanning, hair over the abdomen was clipped, the skin surface was cleaned with 70% isopropyl alcohol, and coupling gel was applied.

2.5 | Two-dimensional (B-mode) ultrasonography

The abdomen was scanned by B-mode ultrasonography using a broadband curved array transducer (5-8 MHz) and the GI tract also was scanned using a broadband linear array transducer (3-9 MHz). The ultrasonographic appearance of the duodenum was evaluated for the following criteria: wall thickness,²⁴ wall layering, motility, regional lymphadenopathy (the reference range used for thickness was 5-8 mm, depending on dog size),²⁵ echogenicity changes of mesentery, and presence of fluid, using a modification of a previously described scoring system.^{26,27} Briefly, the ultrasonographic score was expressed, based on the number of alterations on US examination, as normal (no alteration, 0 point), mild (1-2 alterations, 1 point), moderate (3-4 alterations, 2 points), and severe (≥ 5 alterations, 3 points).

2.6 | Contrast-enhanced ultrasonography

Dogs were placed in left lateral recumbency. The descending duodenal loop was imaged at the level of the right kidney for the CEUS. The loop was scanned in a transverse section and the broadband linear array transducer remained static during the study. Contrast-specific software (Pulse Inversion harmonic and power Modulation combined, Philips Healthcare) with a low mechanical index set at 0.07 and was activated. The gain setting was regulated to obtain a completely anechoic duodenal wall with the only possible exception being the hyperechoic serosal layer and central hyperechoic line arising from the bowel lumen. A bolus injection

(0.05 mL/kg) of contrast medium (Sonovue, Bracco diagnostic, Milano, Italy) was manually made through an indwelling cephalic venous catheter (20 or 22G), immediately followed by a rapidly administered bolus of 4 mL saline. The images were recorded as cine-segments in Digital Imaging and Communications in Medicine (DICOM) format of 60 seconds and then transferred to a personal computer. All studies were anonymized and 2 independent investigators (N. Linta and A. Diana) performed quantitative analysis blinded to any information about the dog (IBD group, control group, or treatment group at T0 or T1).

A DICOM ultrasound viewer (Show Case software, Trillium Technology, Ann Arbor, Michigan) was used to review the images and to export selected frames for qualitative analysis. The distribution of the contrast medium within the duodenal wall was evaluated subjectively by 1 investigator (N. Linta) as satisfactory or unsatisfactory based on the degree of duodenal mural enhancement and homogeneity of mural enhancement at peak intensity (PI). The pattern of contrast enhancement also was described and compared to what was reported previously in healthy dogs.¹³⁻¹⁵

Commercial software (QLAB, Version 9.1, Advanced Quantification Software, Philips Healthcare) was used for quantitative and computerized analysis of the contrast medium blood pool phase. A region of interest (ROI), drawn to cover the widest portion of the intestinal section, was placed manually in the duodenal wall. The ROI was maintained in the same position using an automatic motion compensation tool. This tool prevented displacement of the ROI during respiratory motion. Furthermore, the ROI was adjusted manually on those frames that were severely affected by respiratory motion. Artifactual data from adjacent tissue that moved into the ROI during respiratory motion or gas bubbles were manually removed from the final data set to minimize noise. The raw data obtained from each dog were plotted as quantitative time-intensity curves after the data were fitted to a linear mathematical model curve. From these curves, the following perfusion variables were calculated: arrival time (AT, expressed in seconds), time to peak from injection (TTP), expressed in seconds, time to peak from initial rise (TTPinr, expressed in seconds), peak intensity (PI, expressed in arbitrary units [au]), mean transit time (MTT, expressed in seconds), area under the curve (AUC, expressed in au seconds), and wash-in rate (Wi, expressed in au/s).

2.7 | Statistical analysis

The distribution of data was assessed using the D'Agostino-Pearson's test. Normally distributed data were reported as mean \pm SD, and non-normally distributed data were expressed as median and range (minimum and maximum). For each CEUS variable measurement, intraobserver and interobserver variability was determined using the intraclass correlation coefficient (ICC). In particular, the same investigator (N. Linta) and the other investigator (A. Diana) repeated the CEUS measurements in 25% and all dogs, respectively. Intraclass correlation coefficient values were categorized as poor (<0.50), fair (0.50-0.70), good (0.70-0.90), and excellent (0.90-1.0). Comparison of continuous variables between the IBD and the control group as well as between the IBD subgroups, divided according to CCECAI, and control group was carried out using an

unpaired Student's *t* test or Mann-Whitney test, as needed. Comparisons of the ultrasonographic and CEUS variables, CCECAI, and endoscopic and histological scores in dogs with IBD before and after treatment were done using the Student's *t* test for paired data or the Wilcoxon matched 2-tailed test for repeated measurements.

Receiver operating characteristic (ROC) curves were obtained by plotting sensitivity (Se) vs specificity (Sp) to determine the ability of those CEUS parameters showing significant differences between healthy dogs and dogs with IBD, and between healthy dogs and dogs with severe IBD. The AUC of ROC curves were obtained and the optimal cutoffs corresponding to the values closest to the upper left corner of the graph were identified (Youden criterion). The value of AUC as a criterion of the accuracy of the tested indices was defined as low (0.5-0.7), moderate (0.7-0.9), or high (>0.9).²⁸

Spearman rank correlation analysis was performed to assess if CEUS variables and the CCECAI, B-mode ultrasonographic, endoscopic, and histological scores of the IBD group were significantly correlated. All statistical analyses were performed using commercially available software (Microsoft Excel, Windows 7, Microsoft, Redmond, Washington; Microsoft Office Professional Plus 2013, Microsoft Corporation, Bellevue, Washington; Prism 5, GraphPad Software Inc, San Diego, California; MedCalc, MedCalc Software Ltd, Ostend, Belgium). Significance was set at $P < .05$.

3 | RESULTS

3.1 | Animals

Forty-two dogs were included in the IBD group. Mixed breed dogs were overrepresented (23.8%, 10/42 dogs) and the predominant breed was German Shepherd dog (16.7%, 7/42 dogs). There were 30 intact and 4 neutered males, and 3 intact and 5 neutered females. The mean age was 4.7 ± 3.4 years. Twenty dogs were included in the control group. Mixed breeds were overrepresented (25%, 5/20 dogs) and the predominant breed was German Shepherd dog (20%, 4/20 dogs). There were 10 neutered and 4 intact females, and 5 intact and 1 neutered male. The mean age was 5.5 ± 2.2 years.

3.2 | Clinicopathological findings, clinical score, endoscopic score, and histologic score

Main abnormalities noted on hematological and biochemical tests in the IBD group were alterations in serum folate (median, 10.3 $\mu\text{g/L}$ [1.72-24]; reference interval, 6.5-11.5 $\mu\text{g/L}$), cobalamin (314 ng/L [69-1500]; reference interval, 250-730 ng/L), and CRP (0.1 mg/dL [0.01-6.29]; reference interval, 0-0.5 mg/dL) concentrations. In particular, 37.8% (14/37) of dogs had decreased folate concentrations and 43.2% (16/37) had increased folate concentrations. In 43.2% (16/37) of dogs, the cobalamin concentrations were decreased, whereas in 16.2% (6/37) they were increased. In 51.5% (17/33) of dogs, CRP concentrations were increased.

The median CCECAI score of IBD group was 6.5 (2-12). In particular, CCECAI was normal, mild, moderate, severe, and very severe in 7, 10, 14, 8, and 3 dogs, respectively. In the treatment group, median CCECAI score was significantly decreased at T1 (3 [0-9]) compared to that at T0 (7.5 [4-11], $P = .007$).

The median endoscopic and histologic scores of the IBD group were 2 (0-6) and 11 (6-20), respectively. In the 12 dogs of treatment group, median endoscopic and histologic scores at T0 were 3.5 (1-5) and 11 (8-16), respectively, and no significant difference was found at T1 for endoscopic and histologic scores, 2 (1-6; $P = .15$) and 11 (3-15; $P = .25$), respectively.

3.3 | Ultrasound procedures and analysis

3.3.1 | Bidimensional (B-mode) ultrasonography

In the IBD group, B-mode US examination showed duodenal wall thickening in 50% of dogs (21/42) with a mean thickness of 4.3 ± 1.3 mm and altered wall layering in 73.8% of dogs (31/42 dogs). Altered wall layering was characterized by mucosal hyperechoic spots, mucosal hyperechoic lines, prominence of the submucosal layer, and diffuse increased mucosal echogenicity in 29.0% (9/31), 51.6% (7/31), 9.7% (3/31), and 67.7% (21/31) of dogs, respectively. Other US findings included altered intestinal motility (either increased or decreased), hyperechogenicity of the mesentery, regional lymphadenomegaly (mean size 9.40 ± 4.36 mm), and abdominal effusion in 19.0% (8/42), 30.9% (13/42), 38.1% (16/42), and 28.6% (12/42) of dogs, respectively. The median ultrasonographic score of the IBD group was 2 (0-6).

In the 12 dogs of the treatment group, the median ultrasonographic score did not change significantly from T0 (1 [0-6]) to T1 (1 [0-4]; $P = .36$).

3.3.2 | Contrast-enhanced ultrasonography

Contrast medium enhancement of the duodenal wall was subjectively judged satisfactory in all dogs of the control group and dogs with IBD at both time points (T0 and T1). During CEUS, branches of the pancreatic-duodenal artery were clearly identified and there was a simultaneous radial enhancement of the duodenal wall from the mesenteric and antimesenteric sides. At PI, the duodenal wall showed homogeneous contrast medium distribution with lack of delineation of each intestinal wall layer followed by a gradual and slow washout of contrast medium from the intestinal wall. This pattern of contrast enhancement was present in all 3 groups of dogs (control group, IBD group, and treatment group; Figure 1).

The intraobserver ICCs were excellent, ranging from 0.98 (TTPinr) to 1 (AT, TTPinj, MTT, and Wi). The interobserver ICCs were excellent (ranging from 0.92 to 0.98) for all CEUS variables except for TTPinr for which ICC was good (0.74). Quantitative analysis of contrast medium perfusion of the duodenal wall was performed in 50% (10/20), 85.7% (36/42), and 91.7% (11/12) of dogs

in the control group, IBD group, and treatment group, respectively. Excessive respiratory motion or peristaltic activity precluded quantitative analysis in the remaining dogs. Tables 1 and 2 summarize the comparison of CEUS variables between the IBD group and control group and between each IBD subgroups and the control group. In particular, only the median of PI and AUC was significantly increased in the IBD group compared to that of the control group ($P = .03$ for both comparisons). Comparing CEUS perfusion parameters between the control group and the 4 CCECAI groups, PI, MTT, AUC, and Wi values were significantly higher in the dogs of severe IBD subgroup compared to those of control group ($P = .01$, $P = .01$, $P < .01$, and $P = .04$, respectively; Figure 2). In addition, PI and AUC were significantly higher in the IBD group with mild disease than in the control group ($P = .01$, for both comparisons). No significant difference was found for all quantitative CEUS parameters between T0 and T1 in the treatment group (Table 3).

Results of the ROC curve analysis for the CEUS perfusion parameters PI and AUC in discriminating dogs with IBD from clinically healthy dogs are presented in Figure 3. The accuracy of both PI and AUC was moderate with $AUC \pm SE = 0.74 \pm 0.07$ and 0.73 ± 0.07 , respectively. In particular, PI and AUC had a Se of 58.33% (95% confidence interval [CI], 40.76%-74.49%) and Sp of 100% (95% CI, 66.37%-100.0%) at the cutoff value >4.94 au, and a Se of 69.44% (95% CI, 51.89%-83.65%) and a Sp of 66.67% (95% CI, 29.93%-92.51%) at the cutoff value >51.04 au, respectively.

Results of the ROC curve analysis of the CEUS perfusion parameters PI, AUC, MTT, and Wi to discriminate dogs with severe IBD from clinically healthy dogs are presented in Figure 4. The accuracy was high for PI ($AUC \pm SE = 0.94 \pm 0.05$) with a Se of 77.78% (95% CI, 39.99%-97.19%) and a Sp of 100% (95% CI, 66.37%-100.0%) at the cutoff value >4.94 au. The 3 others CEUS parameters AUC, MTT, and Wi showed only moderate accuracy ($AUC \pm SE = 0.89 \pm 0.08$, 0.84

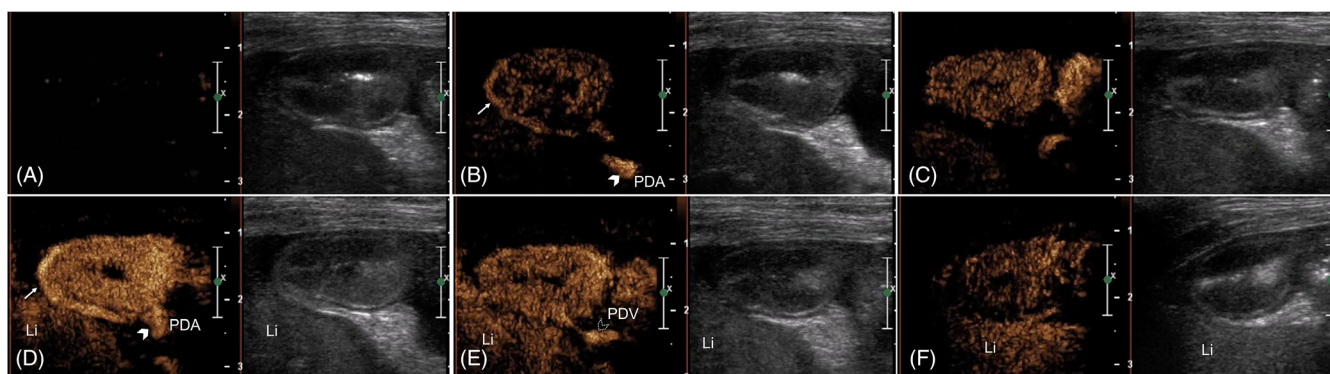


FIGURE 1 A representative contrast-enhanced ultrasound sequence (A-F) of a duodenal wall in a transverse plane after the injection of Sonovue from a dog affected by inflammatory bowel disease. Each image illustrates contrast enhancement on the left and the grayscale image on the right. A, The duodenum immediately before contrast medium injection is shown on the grayscale image. B, Soon after Sonovue injection, the contrast medium is visible in the pancreatic-duodenal artery (PDA) (white arrowhead) and in its branch (white arrow) surrounding the duodenal wall. Some microbubbles are also visible in the duodenal wall. C, A simultaneous radial enhancement of the duodenal wall from the mesenteric and antimesenteric sides is evident. D, At PI, the duodenal wall shows a homogeneous contrast medium transmural distribution with lack of delineation of each intestinal wall layers. Pancreatic-duodenal artery (white arrowhead) and in its branch (white arrow) are still visible. E, F, A gradual decrease of contrast medium from the intestinal wall is seen in the wash-out phase. Pancreatic-duodenal vein (PDV) (white dotted arrowhead) is also visible. PI, peak intensity

TABLE 1 Results of quantitative contrast-enhanced ultrasonography of the duodenum of clinically healthy dogs (control group) and dogs affected by IBD (IBD group)

Perfusion variable	Control group (n = 10)	IBD group (n = 36)	P value
AT (s)	10.86 ± 2.52	11.25 ± 4.86	.82
TTPinj (s)	15.48 ± 2.91	15.96 ± 6.01	.82
TTPinr (s)	4.49 ± 0.95	5.22 ± 1.45	.16
PI (au)	3.85 (1.86-4.93)	5.10 (0.63-15.16)	.03
MTT (s)	9.15 (6.82-12.51)	10.63 (4.50-21.29)	.14
AUC (au s)	47.63 (22.68-62.15)	63.62 (5.31-212.20)	.03
Wi (au/s)	0.80 (0.30-1.30)	0.90 (0.14-3.92)	.5

Note: Normal and not normal distributed data are given as mean ± SD or median and range (minimum-maximum), respectively.

Abbreviations: AT, arrival time; au, arbitrary unit; AUC, area under the curve; IBD, inflammatory bowel disease; MTT, mean transit time; n, number of dogs; PI, peak intensity; TTPinj, time to peak from injection; TTPinr, time to peak from initial rise; Wi, wash-in rate.

TABLE 2 Results of quantitative contrast-enhanced ultrasonography of the duodenum of clinically healthy dogs (control group) and dogs affected by IBD of different clinical severity (IBD minimal IBD subgroup; mild IBD subgroup; moderate IBD subgroup; severe IBD subgroup)

Perfusion variable	Control group (n = 10)	Minimal IBD subgroup (n = 6)	Mild IBD subgroup (n = 10)	Moderate IBD subgroup (n = 11)	Severe IBD subgroup (n = 9)
AT (s)	10.86 ± 2.52	9.99 ± 5.26 [P = .53]	11.20 ± 5.72 [P = .55]	10.95 ± 4.7 [P = .96]	12.51 ± 4.31 [P = .34]
TTPinj (s)	15.48 ± 2.91	14.20 ± 6.45 [P = .46]	16.58 ± 6.14 [P = .97]	16.61 ± 5.69 [P = .6]	15.65 ± 6.76 [P = .95]
TTPinr (s)	4.49 ± 0.95	4.21 ± 1.3 [P = .39]	5.39 ± 1.39 [P = .12]	5.67 ± 1.62 [P = .07]	5.16 ± 1.27 [P = .22]
PI (au)	3.85 (1.86-4.93)	4.13 (0.63-11.84) [P = .78]	5.28 (2.91-11.61) [P = .01]	3.94 (1.27-15.16) [P = .65]	5.69 (4.12-14.39) [P = .01]
MTT (s)	9.15 (6.82-12.51)	8.76 (4.5-17.54) [P = .86]	11.02 (7.92-21.25) [P = .11]	10.73 (4.66-21.29) [P = .65]	10.73 (9.11-19.72) [P = .01]
AUC (au s)	47.63 (22.68-62.15)	56.83 (5.31-153.9) [P = .53]	75.38 (34.82-187.6) [P = .01]	48.70 (17.04-212.2) [P = .59]	67.50 (50.07-190.7) [P = .004]
Wi (au/s)	0.80 (0.30-1.30)	0.86 (0.14-2.87) [P = .86]	0.89 (0.43-3.92) [P = .57]	0.73 (0.16-3.36) [P = .73]	1.06 (0.54-2.54) [P = .04]

Note: Normal and not normal distributed data are given as mean ± SD or median and range (minimum-maximum), respectively.

Abbreviations: AT, arrival time; au, arbitrary unit; AUC, area under the curve; IBD, inflammatory bowel disease; MTT, mean transit time; n, number of dogs; PI, peak intensity; TTPinj, time to peak from injection; TTPinr, time to peak from initial rise; Wi, wash-in rate.

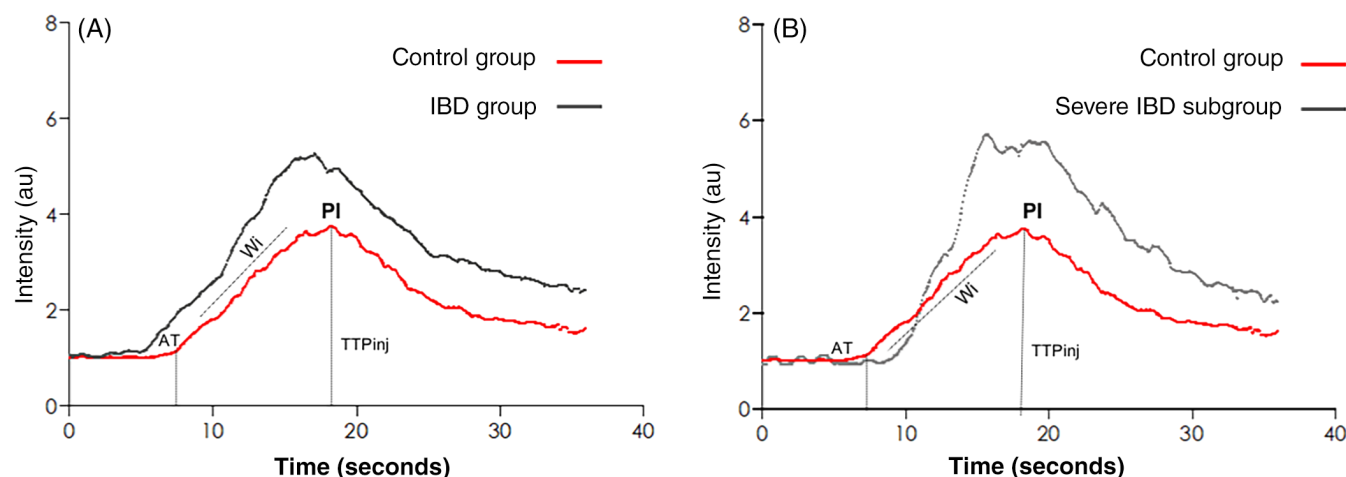


FIGURE 2 Average time-intensity curves of duodenal wall from clinically healthy dogs (control group) (red line) and dogs affected by IBD (IBD group) (black line) (A) and from clinically healthy dogs (control group) (red line) and dogs with severe IBD (severe IBD subgroup) (gray line) (B). Contrast enhancement from the duodenal wall increases until reaching peak intensity during the arterial phase. AT, arrival time; au, arbitrary units; IBD, inflammatory bowel disease; PI, peak intensity; TTPinj, time to peak from injection; Wi, wash-in rate

± 0.09, and 0.75 ± 0.12, respectively). In particular, AUC had a Se of 88.89% (95% CI, 51.75%-99.72%) and a Sp of 77.78% (95% CI, 39.99%-97.19%) at the cutoff value >52.26 au; MTT had a Se of 77.78% (95% CI, 39.99%-97.19%) and a Sp of 77.78% (95% CI, 39.99%-97.19%) at the cutoff value >9.48 seconds, and Wi had a Se of 77.78% (95% CI, 39.99%-97.19%) and a Sp of 77.78% (95% CI, 39.99%-97.19%) at the cutoff value >0.9 au.

Correlation analyses between CEUS perfusion parameters and CCECAI, ultrasonographic, endoscopic, and histological scores showed no significant correlation for any of them.

4 | DISCUSSION

We described the duodenal CEUS pattern of dogs with IBD and compared it to that of healthy dogs and in the same dogs after treatment. The contrast enhancement pattern in the duodenal wall was similar to what was previously described in dogs.^{13-16,21} In particular, after an initial visualization of the small branches of the pancreatic-duodenal artery, a progressive radial and simultaneous uptake of contrast medium from the entire intestinal wall was evident and reached a homogeneous intensity peak followed by a gradual washing out of

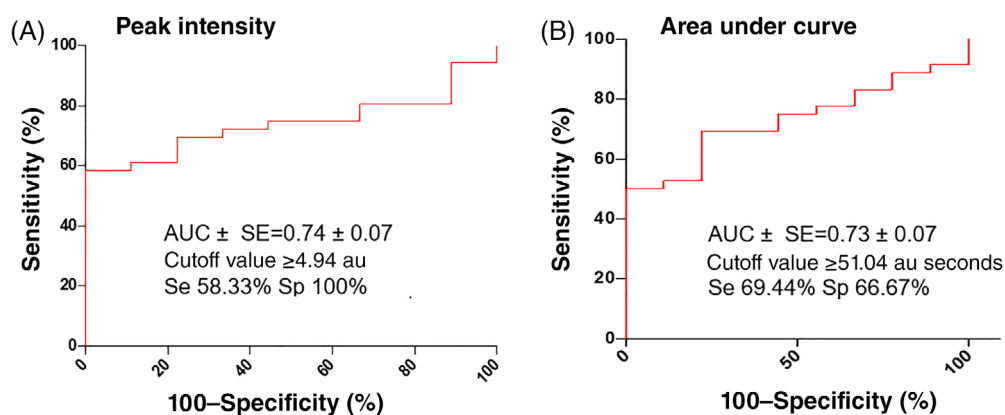
TABLE 3 Results of quantitative contrast-enhanced ultrasonography of the duodenum of dogs of the treatment group at two time points (T0 and T1)

Perfusion variable	T0 (n = 10)	T1 (n = 10)	P value
AT (s)	11.31 ± 2.72	11.75 ± 4.060)	.71
TTPinj (s)	17.46 ± 3.61	16.96 ± 4.72	.85
TTPinr (s)	5.86 ± 1.49	5.21 ± 0.10	.19
PI (au)	4.25 (1.27-7.32)	5.75 (2.69-12.34)	.16
MTT (s)	9.49 (4.66-12.93)	9.71 (4.47-15.94)	1
AUC (au s)	53.73 (17.04-81.58)	63.23 (32.84-158.7)	.23
Wi (au/s)	0.54 (0.16-1.57)	1.08 (0.32-2.48)	.16

Note: Normal and not normal distributed data are given as mean ± SD or median and range (minimum-maximum), respectively.

Abbreviations: AT, arrival time; au, arbitrary unit; AUC, area under the curve; MTT, mean transit time; n, number of dogs; PI, peak intensity; TTPinj, time to peak from injection; TTPinr, time to peak from initial rise; Wi, wash-in rate.

FIGURE 3 Receiver operating characteristics curves of the CEUS parameters peak intensity (A) and area under the curve (B) for distinguishing dogs with inflammatory bowel disease (IBD group) from clinically healthy dogs (control group). AUC, area under the curve; CEUS, contrast-enhanced ultrasonography; IBD, inflammatory bowel disease; Se, sensitivity; Sp, specificity



contrast medium. This aspect may be attributed to the typical blood flow distribution within the intestinal wall of dogs in which the mucous layer receives a higher percentage of arterial blood flow (92.8%) than does the submucosal layer (0.77%).²⁹ In our study, no difference in the enhancement pattern between healthy dogs and those with IBD was observed. A similar result also was reported previously where subjective observations of duodenal contrast enhancement after contrast injection showed no obvious differences in dogs in the remission chronic inflammatory enteropathy, symptomatic chronic inflammatory enteropathy, or intestinal lymphoma groups compared to the control group.²¹

In humans, many studies have reported the use of the CEUS pattern to distinguish the active phase of Crohn's disease, an intestinal disorder resembling IBD in dogs.^{8,10,26,30}

Four wall enhancement patterns have been identified in humans with Crohn's disease: complete wall enhancement (single-layer pattern), enhancement of the inner layers except the muscular layer (bi-layered pattern), enhancement of only the submucosal layer (tri-stratified pattern), and complete absence of enhancement.⁸ In particular, the 2 former patterns correspond to the phase of active inflammation, whereas the latter 2 patterns were typical of the quiescent phase characterized by intraparietal fibrosis.^{8,10,30} In all of our dogs with IBD, we identified a single distribution pattern of contrast medium characterized by complete wall enhancement. This feature presumably

is related to an inflammatory condition of the wall without fibrosis, confirmed by histopathology.

Concerning quantitative wall perfusion, the employed dose of contrast medium (0.05 mL/kg) appeared satisfactory in all dogs of the 3 groups. Furthermore, results of ICC showed excellent intra- and inter-observer repeatability of CEUS quantitative measurements. Our results of perfusion variables of healthy dogs cannot be fully compared to those reported in previous studies^{13-16,21} because several factors can influence quantitative CEUS parameters. First, our animals were manually restrained without sedation, which can influence splanchnic perfusion and modify quantitative parameters.^{17,31} The type and dosage of contrast medium, ROI position, and also type of intestinal pathology (ie, IBD) in our study were different from those previously reported.^{13-15,21}

Comparing the CEUS perfusion parameters between healthy animals and dogs with IBD, all CEUS parameters were higher in dogs with IBD, although statistically significant differences only were observed for PI and AUC. Similar results also were found in a previous study that showed a significant increase of PI and AUC in dogs with symptomatic chronic inflammatory enteropathy.²¹

Peak intensity and AUC of CEUS are indicators of regional blood volume of the mucosa, and the degree of enhancement reflects the vascularity of the inflamed intestinal wall. In severely affected dogs based on CCECAI scores, 2 other CEUS parameters (ie, MMT and Wi) were significantly different. Human patients with Crohn's disease

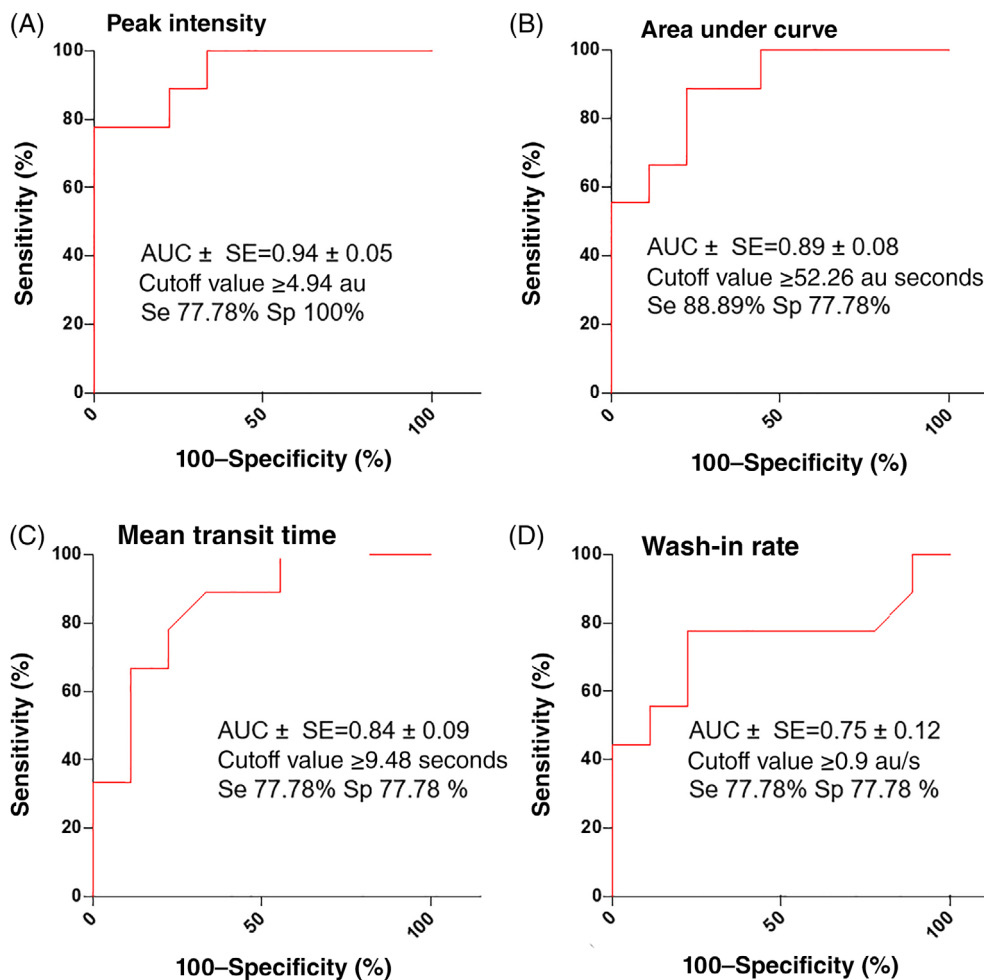


FIGURE 4 Receiver operating characteristics curves of the CEUS parameters peak intensity (A), area under the curve (B), mean transit time (C), and wash-in rate (D) for distinguishing dogs with severe inflammatory bowel disease (severe IBD subgroup) from clinically healthy dogs (control group). AUC, area under the curve; CEUS, contrast-enhanced ultrasonography; IBD, inflammatory bowel disease; Se, sensitivity; Sp, specificity

have increased intestinal blood flow, most likely because intestinal inflammation induces both increased macro-vasculature and neo-angiogenesis of intraparietal microvessels.^{8,30,32} Although we found significant differences for only 2 variables, we hypothesize that the higher values of perfusion parameters obtained in dogs with IBD also may be caused by inflammation.

The diagnostic accuracy of CEUS perfusion parameters in differentiating healthy dogs from those with IBD was only moderate. In particular, PI and AUC, at the cutoff values of 4.94 au and 51.04 au, respectively, achieved the best compromise between Se (58% and 69.44% for PI and AUC, respectively) and Sp (100% and 66.67% for PI and AUC, respectively). Considering the IBD dogs with severe disease compared to the control group, accuracy was higher for PI with a Se and a Sp of 77.78% and 100%, respectively, at the cutoff value >4.94 au.

In human medicine, the accuracy of PI in distinguishing quiescent from active Crohn's disease is high, reaching values for Se and Sp of 89% and 93%, respectively.¹² The lower accuracy found in our study could be related to the relatively smaller study population, the lack of fibrosis in the duodenal wall of dogs with IBD compared to humans with Crohn's disease, and the different aims of the study (ie, differentiating healthy dogs from sick animals instead of differentiating active from the inactive disease).

In our study, we did not find a correlation between CEUS perfusion parameters and the other scores (ie, CCECAI, ultrasonographic, and endoscopic or histologic scores) of dogs affected by IBD. These results are in contrast to those previously reported in humans with Crohn's disease that showed a positive correlation between perfusion parameters and clinical, endoscopic, and histological scores.^{10,11,33} These differences could have occurred for several reasons: differences in the relative size of the study population, the aim of discerning active from inactive disease in studies of humans rather than healthy from sick patients as in our cases. Finally, the absence of a fibrotic component in the duodenal wall in dogs with IBD detected on histology was notable.

In our study, we also compared CEUS parameters and CCECAI, B-mode ultrasonography, and endoscopic and histologic scores in dogs with IBD before (T0) and after a period of 62 to 68 days (T1) during which the dogs received standardized treatment. This time lapse was chosen based on a previous study in which clinical and ultrasonographic features of dogs affected by IBD were compared before and after standardized treatment.²⁷ The only significant difference was found for CCECAI score in dogs before and after treatment. This result agrees with what was previously reported in the veterinary literature and confirms the usefulness of the CCECAI score in monitoring the response to treatment in dogs with IBD.²²

We did not find significant differences in CEUS qualitative and quantitative analysis between the 2 different T0 and T1 in dogs with IBD. These results differ from those reported in human patients with Crohn's disease, where PI and AUC were significantly decreased after appropriate treatment.^{10-12,33} The lack of difference in CEUS parameters in our dogs with IBD pre- and post-treatment could be a consequence of the smaller study population or the shorter time interval after treatment or both.

Our study had some limitations. First, the population of control dogs prospectively recruited was considered healthy on the basis of clinical, laboratory, and ultrasonographic findings. No GI endoscopy or surgical biopsies were performed and histological confirmation of the normality of the duodenum was not available.

Second, for the quantification of perfusion variables, ROI were manually drawn and positioned on the duodenal wall. The lack of a standardized size of ROI could have affected the results of the perfusion parameters. Finally, excessive respiratory motion or peristaltic activity or both precluded quantitative analysis in some dogs, decreasing the sample size of the groups.

In conclusion, we confirmed the feasibility of CEUS for the evaluation of duodenal wall perfusion in dogs with IBD. Specific perfusion parameters (ie, PI and AUC) showed clinical usefulness to discriminate dogs with IBD from clinically healthy dogs. No significant differences for CEUS perfusion parameters were found to differentiate dogs with IBD from those receiving standardized treatment. Because of the small number of dogs available for follow-up after treatment, these latter results require further validation in a larger number of dogs.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict 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

Approved by the Ethical Committee of the University Teaching Hospital (No. 28-IX/9). Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Pascaline Pey  <https://orcid.org/0000-0003-3917-4756>

Marco Baron Toaldo  <https://orcid.org/0000-0001-9609-1856>

Marco Pietra  <https://orcid.org/0000-0003-4431-0791>

Giuliano Bettini  <https://orcid.org/0000-0003-1857-7329>

Alessia Diana  <https://orcid.org/0000-0003-1709-3920>

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