Correlation of minimally invasive imaging techniques to assess intestinal mucosal perfusion with established markers of canine chronic inflammatory enteropathy

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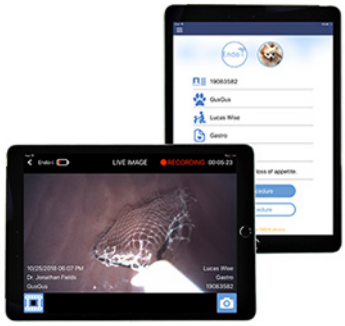

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Correlation of minimally invasive imaging techniques to assess intestinal mucosal perfusion with established markers of chronic inflammatory enteropathy in dogs

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Abstract

Background: Minimally invasive diagnostic imaging techniques to detect intestinal inflammation in dogs are lacking. Contrast-enhanced ultrasound (CEUS) and endoscopic narrow band imaging-like endoscopy (Storz Professional Image Enhancement System [SPIES]) might allow quantification of intestinal mucosal perfusion and microvessel density in chronic inflammatory enteropathy (CIE) of dogs.

Hypothesis/Objectives: Markers of mucosal perfusion as determined by CEUS and SPIES endoscopy are potentially useful diagnostic markers to help characterize CIE and correlate with histological inflammation type and severity.

Animals: Thirty client-owned dogs diagnosed with CIE at a referral hospital were prospectively enrolled.

Materials and Methods: Data from CEUS, SPIES, and white light (WL) endoscopy were correlated with World Small Animal Veterinary Association (WSAVA) endoscopy and histology scores and vessel density as determined by immunohistochemistry for von Willebrand factor (vWF). Automated linear modeling was used to determine predictors of endoscopic and histologic severity.

Results: Duodenal histology correlated with SPIES data (area percentage value, *rho* = 0.424, *P* = .04). White light endoscopy parameters correlated with SPIES parameters in the duodenum. Colonic histology correlated positively with CEUS, whereas colonic CEUS parameters correlated inversely with vWF expression. Several duodenal parameters combined predicted duodenal histology scores to a level of 73.4%. For the colon, 2 parameters contributed more than others to 73.1%.

Conclusion and Clinical Importance: Minimally invasive CEUS and SPIES appear feasible to assess intestinal perfusion in CIE. Use of SPIES endoscopy may be promising for assessing small intestinal inflammation, whereas CEUS could be used to assess

Abbreviations: APV, area percentage value; AUC, area under the curve; BW, black-and-white; CD, Crohn's disease; CEUS, contrast-enhanced ultrasound; CIE, chronic inflammatory enteropathy; DAB, 3,3′-diaminobenzidine; GI, gastrointestinal; IBD, inflammatory bowel disease; IDV, integrated density value; IHC, immunohistochemistry; NBI, narrow-band imaging; RawID, raw intensity density; ROI, region of interest; SBP, systolic blood pressure; SPIES, Storz Professional Image Enhancement System; TBS, tris-buffered saline; TTP, time to peak; TVA, total vesselness area; UC, ulcerative colitis; vWF, von Willebrand factor; WL(E), white light (endoscopy); WSAVA, World Small Animal Veterinary Association.

colonic perfusion and inflammation. Both techniques need to be investigated further for their clinical utility.

KEYWORDS
immunohistochemistry, inflammatory bowel disease, perfusion, ultrasound, vessels, von Willebrand factor

1 | INTRODUCTION

Minimally invasive or noninvasive diagnostic imaging tests to assess the extent and type of inflammation in the intestine of dogs are lacking. Biopsy of the gastrointestinal (GI) tract still is and considered the ideal method by which to assess both inflammatory infiltrates and architectural and morphological changes in the intestinal mucosa.[1] However, histopathology of endoscopic mucosal biopsy specimens, which is the method most commonly employed in dogs with chronic inflammatory enteropathy (CIE),[1] is problematic because it depends on the skill level of the endoscopist. In addition, there is lack of agreement among pathologists,[2] even when using well-established[3] or simplified[4] grading systems.

Based on the 5 cardinal signs of inflammation (*calor*, *rubor*, *dolor*, *tumor*, *functio laesa*), changes in capillary vessel density, dilatation (flow), and permeability may be used as a proxy to assess the presence or severity of focal tissue inflammation. In addition, angiogenesis and intestinal microcirculation play crucial roles in the pathogenesis of inflammatory bowel disease (IBD) in humans,[5] where they are considered a largely unstudied component in both active Crohn's disease (CD) and ulcerative colitis (UC).[6] This link has not been established or investigated in CIE. With the availability of more advanced imaging tools used in the diagnostic evaluation of chronic GI conditions in dogs (ie, contrast-enhanced ultrasound [CEUS] and endoscopy techniques similar to narrow-band imaging [NBI]), less invasive quantification of intestinal mucosal perfusion and microvessel density or morphology now is possible in dogs.

Contrast-enhanced ultrasound uses gas-filled microbubbles (usually sulfur hexafluoride) as a tracer to assess tissue perfusion.[7,8] The microbubbles remain entirely within the intravascular space and have rheology similar to that of red blood cells.[7] Excess sulfur hexafluoride is exhaled, and the phospholipid microbubble shell enters the endogenous phospholipid metabolic pathway. The microbubbles have an elimination half-life of approximately 6 minutes, and >80% of the administered gas is exhaled via the lungs after 11 minutes.[7] Contrast-enhanced ultrasound has been described as a safe, readily available, inexpensive, and effective tool for surveillance of mucosal healing in people with IBD.[9] It is a safe diagnostic modality in small animals,[10] and already has been used to evaluate abnormalities in several organs of the dog (eg, focal splenic lesions,[11] adrenal tumors,[12] prostatic disease[13]), and the intestine has been assessed using CEUS in healthy dogs[8] and cats.[14,15] In addition, CEUS has been used to assess duodenal perfusion in dogs with CIE and intestinal lymphoma.[16] In humans with IBD, CEUS parameters correlate with CD clinical activity,[17,18] endoscopic disease activity scores,[19] or both,[20] as well as with histological severity of inflammation[21] and histological vessel density.[22]

Narrow band imaging is a novel endoscopic technology that highlights mucosal surface structures and microcapillaries. Optical filters achieve sequential green and blue illumination, narrowing the bandwidth of spectral transmittance, and thus obtaining tissue illumination at selected narrow wavelength bands to achieve the most contrast between vascular structures and the surrounding mucosa.[23] A similar result can be achieved using live image manipulation during acquisition, as employed by some manufacturers, for example, with the Storz Professional Image Enhancement System (SPIES). This system uses image processing to achieve an NBI-like effect after image recording with white light. Direct comparison of NBI with SPIES showed strong agreement of both imaging procedures,[24] but this arrangement so far has not been confirmed in dogs. Narrow band imaging initially was used to facilitate detection of colorectal neoplasia in human patients with and without concomitant IBD, for which it has superior accuracy compared to conventional colonoscopy.[25] It also has been used to characterize inflammatory intestinal lesions,[26] assess mucosal vascular patterns,[27] and identify angiogenesis[5,28,29] in humans with IBD. Neither NBI nor SPIES have been systematically used in GI endoscopies of small animals. In a single case report, a similar technique (blue-green endoscopy) facilitated identification of lesions in the esophagus and stomach of a dog.[30]

Routine histological examination of endoscopic mucosal pinch biopsy specimens from the GI tract does not involve assessment of vessel density, and vessel density is not included in any current guidelines or grading systems for small animals, because it is not used for differentiation between healthy and inflamed intestinal tissue.[3,4] However, immunohistochemistry (IHC) for factor VIII-related antigen/von Willebrand factor (vWF) is a reliable tool to identify endothelial cells in human and canine formalin-fixed paraffin-embedded tissues.[31,32] Increased intestinal mucosal vascularization is a finding typical of active UC and CD in people using this and other methods.[5,6]

We hypothesized that parameters from CEUS and SPIES would correlate with well-established methods to characterize mucosal inflammation (ie, World Small Animal Veterinary Association [WSAVA] endoscopy and histology scores) and would be mirrored by increased capillary density (so-called "vesselness") not only using these diagnostic imaging techniques, but also with histological assessment of vessels within intestinal tissue. Our aims hence were to assess if CEUS and SPIES would allow further characterization of inflammation in dogs with CIE and have the potential to serve as a surrogate, substitute, or novel diagnostic marker of intestinal inflammation in dogs. To

achieve this aim, correlation analysis of quantitative CEUS and SPIES endoscopy using standard endoscopy scores, histologic lesion scores, and vessel density (as determined by vWF IHC) in mucosal samples from dogs diagnosed with CIE was performed.

2 | MATERIALS AND METHODS

Dogs presented to our hospital's internal medicine referral service and suspected to have CIE based on compatible clinical signs (eg, vomiting, diarrhea, weight loss, or some combination of these of ≥3 weeks duration) and exclusion of other potential causes based on comprehensive laboratory diagnostic testing (CBC, serum biochemistry, fecal flotation, urinalysis, baseline serum cortisol concentration or ACTH stimulation test, pre- and post-prandial bile acid serum concentrations, where appropriate), screening for pancreatic disease (trypsin-like immunoreactivity, canine pancreatic lipase) or intestinal malabsorption (serum folate and cobalamin concentrations) and abdominal ultrasound examination were prospectively enrolled between July 2017 and July 2019. Institutional ethical approval was obtained (Veterinary Ethical Review Committee number VERC 21.17), as was individual owner consent. All dogs with a final diagnosis of CIE for which owner consent was obtained were included. Dogs with a suspected or final diagnosis of protein-losing enteropathy were excluded. Control dogs were not included because of lack of ethical approval to perform CEUS and NBI endoscopy in otherwise healthy animals. No sample size calculation was performed given the lack of previous data using these techniques in this configuration and combination in dogs. The number of dogs included was determined by financial coverage for the study allowed by a small grant.

Contrast-enhanced ultrasound of the duodenum and colon was performed at the same time as the routine diagnostic ultrasound examination. Before imaging, noninvasive systolic arterial blood pressure (SBP) was measured in all dogs using Doppler technique. Only dogs with normal SBP (>90 mm Hg and ≤140 mm Hg) that would tolerate ultrasound examination unsedated or sedated with butorphanol alone (0.2 mg/kg body weight IV, repeated once if necessary) were included. This approach was used to avoid any effect of SBP or sedation on local tissue perfusion.[33] Dogs did not receive enemas before abdominal ultrasound or CEUS examinations. All CEUS examinations were performed using the same ultrasonography machine and software (MyLab Twice Esaote, Genova, Italy). A second-generation ultrasound contrast agent consisting of sulfur hexafluoride microbubbles (SonoVue, Bracco Imaging, Milan, Italy) was administered for CEUS imaging (2 mL IV bolus) after standard sagittal plane scans of the duodenum and colon were obtained. From the moment of contrast injection, a 2-minute video clip of the perfusion pattern was recorded using Contrast Tuned Imaging (Esaote, Genova, Italy). A minimum of 15 minutes washout time was maintained between duodenal and colonic CEUS assessments. The CEUS videos were analyzed as a batch by a single observer (Y.-L. Tan) after recruitment was completed. Dedicated software (Vuebox 7.2, Bracco Imaging, Milan, Italy) was used to determine several numerical parameters related to mucosal perfusion (Table S1).

Routine white light (WL) endoscopy (gastroduodenoscopy and ileocolonoscopy) was performed within 1 to 2 days of the routine ultrasound and CEUS examinations in all dogs as part of their diagnostic evaluation, using either a smaller veterinary videoendoscope (Feline Silver Scope; 5.9 mm outer diameter, 110 cm working length, 2 mm working channel; Karl Storz Endoscopy Ltd, Slough, Berkshire, United Kingdom) for dogs <10 kg body weight or a larger veterinary videoendoscope (Multi-Purpose Silver Scope; 7.9 mm outer diameter, 140 cm working length, 2.8 mm working channel; Karl Storz Endoscopy Ltd) for dogs ≥10 kg body weight. Dogs underwent gastroduodenoscopy (n = 30) or coloileoscopy (n = 28) as deemed appropriate for their predominant clinical signs. Standard image documentation of all segments of the GI tract was done by recording videos and still images where appropriate. Serial images for later side-by-side analysis from the same sections of duodenum and colon were taken using the optimized WL setting, CLARA + CHROMA. The CLARA setting automatically identifies and brightens dark image areas, whereas CHROMA intensifies color contrast levels while retaining natural color perception of the image. The so-called "Spectra B" setting also was used, which changes the effective spectral response in the imaging system, leading to the NBI-like color contrast of SPIES.[34] Numerical endoscopy scores were recorded as part of routine documentation using an established template from the WSAVA. Briefly, this template allows scoring of several defined abnormalities in each segment of the GI tract (eg, granularity, edema) on a numerical scale (0 = normal, 1 = mild, 2 = moderate, 3 = marked).

Both WL and SPIES endoscopy images were analyzed as a batch using a freely available cross-platform application framework for medical image processing, which has been established for analysis of NBI endoscopy images in humans (MeVisLab SDK [noncommercial], MeVis Medical Solutions AG and the Fraunhofer Institute, Bremen, Germany). In this software, a pipeline to quantify both WL and SPIES images comparatively for a parameter called "vesselness," which allows the visualization of capillaries in a black-and-white (BW) format, was created based on a prior study performed in humans[35] (Figure S1). Images then were transformed into 8-bit files, and adjusted for threshold values on a full grayscale space, so that only pixels falling within the boundaries of the automatic threshold were analyzed (ImageJ, Fiji, https://imagej.net/Fiji#Downloads).[36,37] This procedure allows reduction of noise within the BW image.

Histopathological diagnoses for individual dogs were based on tissue assessment by the original pathologist. However, for the purpose of our study, all slides were re-examined (or re-cut if not considered adequate) and scored according to WSAVA guidelines[3] by 1 of 2 board-certified pathologists (S. Smith, L. Morrison). Briefly, numerical scores of both inflammatory and morphological features for duodenum and colon were applied, and added for a cumulative score.

To assess microvessels quantitatively within the intestinal tissue, IHC for vWF was performed on all biopsy specimens (a minimum of 8 biopsy specimens per site from each dog). This procedure was done as a batch using an automated IHC stainer (Leica Bond RX

Autostainer, Leica Biosystems, Milton Keynes, United Kingdom). Antigen retrieval involved using proteinase-K (Proteinase K ready-to-use, cat# S3020, Agilent Technologies Ltd [DAKO], Cheadle, United Kingdom) for 20 minutes. All steps were carried out at room temperature. After 3 tris-buffered saline (TBS) washes, the primary antibody (polyclonal rabbit anti-human Von Willebrand Factor antibody, cat#A0082, Agilent Technologies Ltd [DAKO]) was used at a dilution of 1 : 400 for 30 minutes. This application was followed by additional washes (3, TBS; 1, distilled water), a peroxide block of 15 minutes, another TBS wash (as before), incubation with secondary antibody for 20 minutes and incubation with 3,3′-diaminobenzidine (DAB) chromogen for 10 minutes. Hematoxylin counterstaining was performed using a kit (DAKO EnVision+TM system—HRP labeled polymer, anti-rabbit, cat#K4003, Agilent Technologies Ltd [DAKO]) according to the manufacturer's instructions. Stained slides were digitalized using a slide scanner for later quality control with matching software.[38]

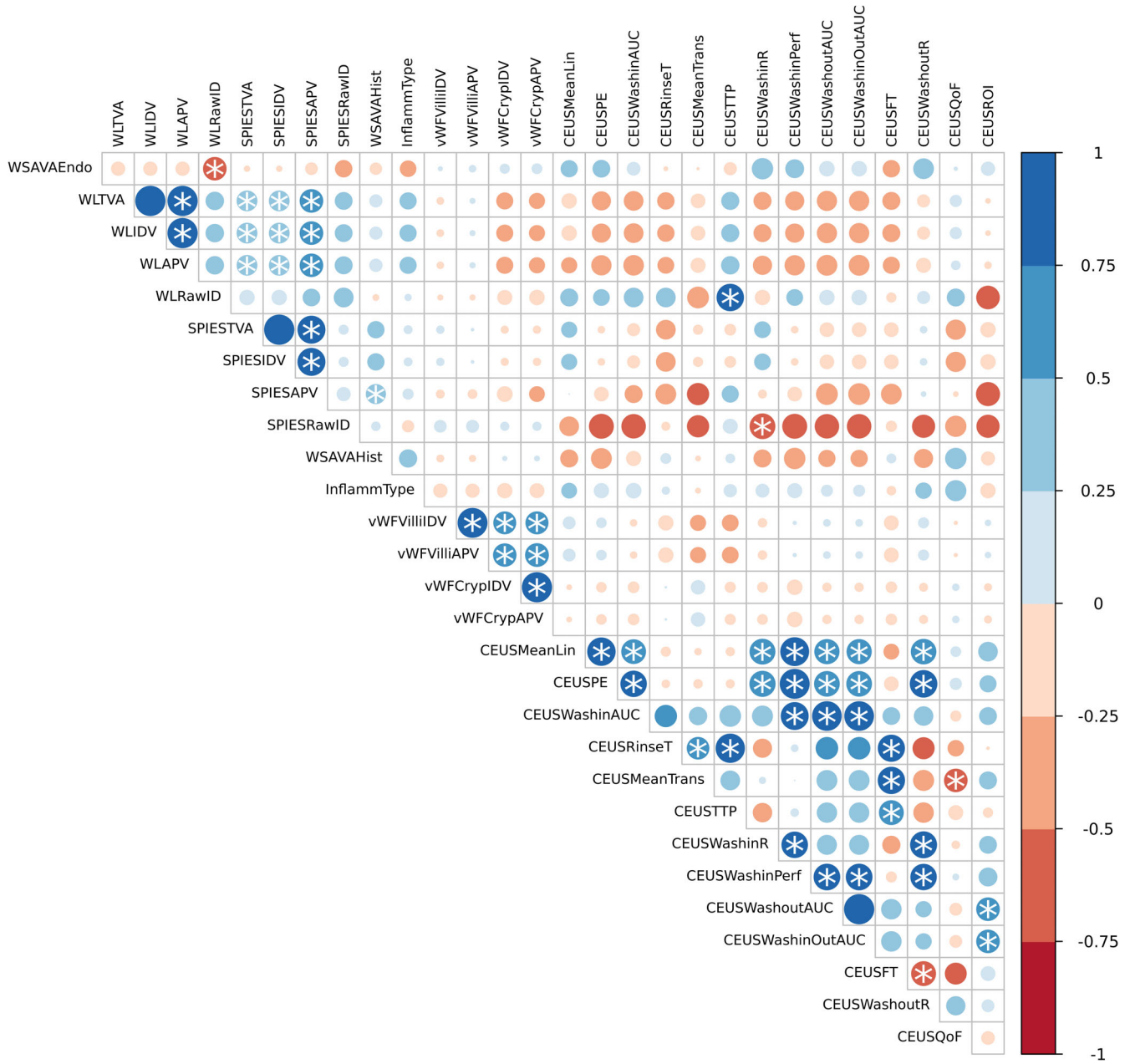

FIGURE 1 Correlation heatmap of duodenal parameters using Spearman's test. A significance level of $P < .05$ is indicated by a white asterisk. The correlation coefficient is represented by the color bar (range from 1 = blue to red = −1), size, and opacity of the circles. APV, area percentage value; AUC, area under the curve; CEUS, contrast-enhanced ultrasound; Endo, endoscopy score; Hist, histology score; IDV, integrated density value; InflammType, histological diagnosis based on type of inflammation; Lin, linearized signal; PE, peak enhancement; Perf, perfusion index; QoF, quality of fit; RawID, raw intensity density; RinseT, rinse time; ROI = region of interest; SPIES, Storz Professional Image Enhancement System; TTP, time to peak; TVA, total vesselness area; vWF, von Willebrand factor; WL, white light; WSAVA, World Small Animal Veterinary Association

Images at ×40 magnification were exported in jpg format for quantification of vWF staining. Five entire images from each of the following sections were analyzed per dog and tissue: longitudinal and cross-sectional images of the duodenal villi, the duodenal crypts, superficial colonic mucosa, and deep colonic mucosa. The IHC image analysis involved automatic quantification of positive staining (as averaged values across the 5 images from each location) using the previously mentioned software (ImageJ). The color convolution tool, which has a presetting for IHC images using DAB and hematoxylin staining, was applied. The image with the DAB staining only was transformed to a BW image as described for the endoscopy images (using ImageJ). For both the endoscopy and IHC images, quantitative parameters of vWF staining were used for analysis. These are standard outputs from IHC from ImageJ, where the total number of pixels of positive staining of the standardized image as well as the percentage of staining of each image were defined as total vesselness area (TVA) and area

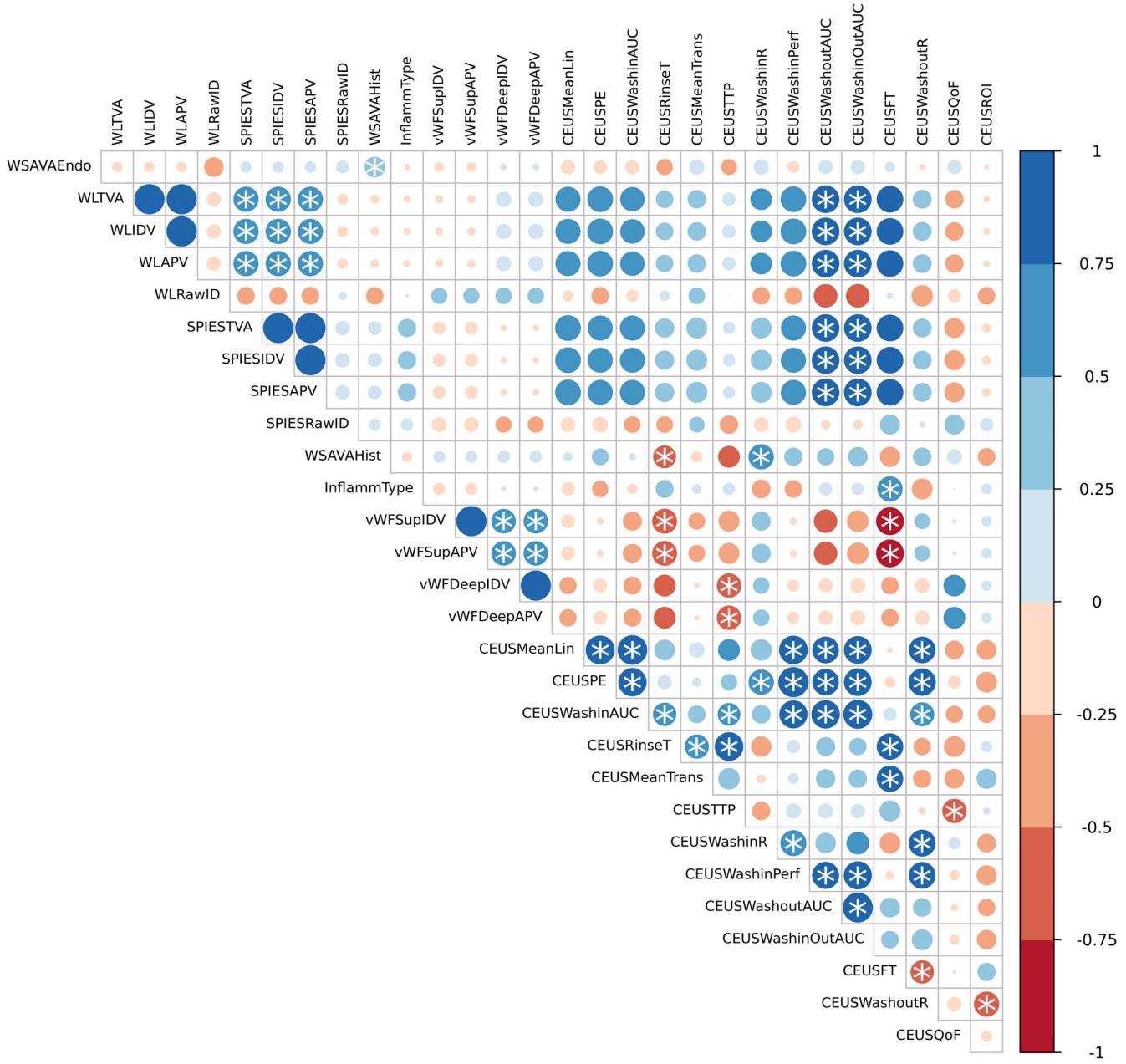

FIGURE 2 Correlation heatmap of colonic parameters using Spearman's test. A significance level of $P < .05$ is indicated by a white asterisk. The correlation coefficient is represented by the color bar (range from 1 = blue to red = −1). APV, area density value; AUC, area under the curve; CEUS, contrast-enhanced ultrasound; Deep, deep areas of colonic tissue; Endo, endoscopy score; Hist, histology score; IDV, integrated density value; InflammType, histological diagnosis based on type of inflammation; Lin, linearized signal; MeanTrans, mean transit time; PE, peak enhancement; Perf, perfusion index; QoF, quality of fit; RawID, raw intensity density; RinseT, rinse time; ROI, region of interest; Sup, superficial areas of colonic tissue; TTP, time to peak; TVA, total vesselness area; vWF, von Willebrand factor; WL, white light; WSAVA, World Small Animal Veterinary Association

percentage value (APV), respectively, whereas the intensity of staining was reported as raw density (RawD) and integrated density value (IDV).

2.1 | Statistical analysis

Statistical analysis was performed using commercial software (IBM SPSS Statistics for Windows, version 24.0, IBM Corp, New York, New York). Categorical data (eg, inflammation type) were converted to a numerical scale for the purpose of analysis. This transformation only was performed to allow software analysis with SPSS using a codification of the text, without any sense of magnitude or cross-category analysis. Data were tested for normality by inspection of histograms where appropriate. Spearman correlation was performed accordingly. A *rho* > 0.4 and <0.6 was considered moderate; >0.6 and <0.8, strong; and >0.8, very strong.[39] A *P* value of <.05 was considered statistically significant. In addition, automated stepwise linear regression modeling was performed using all available data from CEUS, SPIES, and vWF IHC to assess the more complex interactions of data. To do so, 2 different models were created separately for duodenal and colonic data: predictors of WSAVA histology scores and predictors of the endoscopy score. A forward step-wise approach was chosen after comparison with a backwards to mixed stepwise approach based on prediction metrics. Effects with *P* values <.05 were included and effects with *P* values >.05 excluded for these models. Heatmaps of correlation analysis were created using freely available software (R, version 3.5, www.r-project.org).

3 | RESULTS

Thirty pet dogs with CIE were included in the study. The most common breed was Labrador Retriever (n = 7), followed by West Highland White Terrier (n = 4), Border Terrier (n = 3) and 1 each of the following breeds: Bearded Collie, Bulldog (not further classified), Cavalier King Charles Spaniel, English Bull Terrier, Flat-coated Retriever, French Bulldog, German Shepherd dog, Irish Setter, Jack Russell Terrier, Lurcher, Maltese Terrier, Scottish Terrier, and Whippet. In addition, 3 crossbreed dogs were included. Most dogs were male neutered (n = 14), followed by female spayed (n = 8), male intact (n = 4), and female intact (n = 4). Median age was 55 months (range, 5-161 months) and median body weight was 16.2 kg (range, 4.6-31.5 kg).

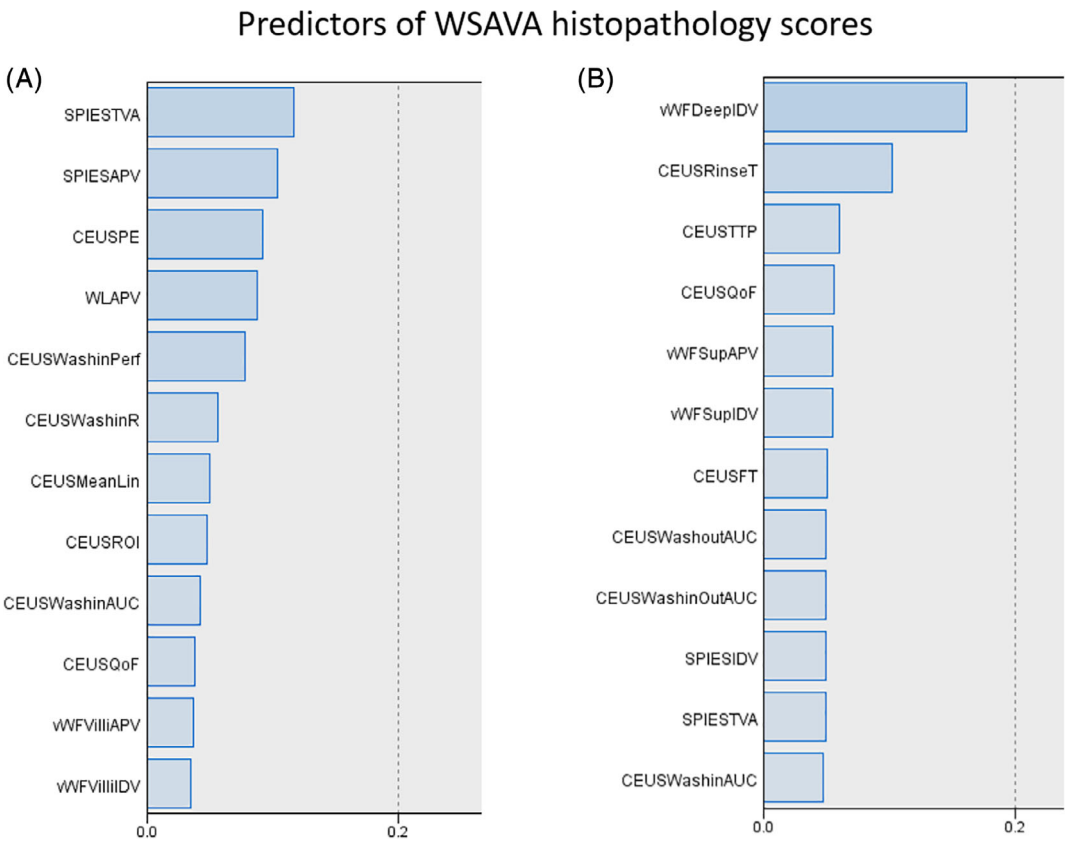

FIGURE 3 Automated stepwise linear regression modeling for factors predicting WSAVA histology score of the duodenum (A) and colon (B). X-axis shows the regression coefficient. APV, area percentage value; AUC, area under the curve; CEUS, contrast-enhanced ultrasound; Col, colon; Deep, deep areas of colonic tissue; Duo, duodenum; IDV, integrated density value; Lin, linearized signal; PE, peak enhancement; Perf, perfusion index; QoF, quality of fit; RawD, raw density; RinseT, rinse time; ROI, region of interest; Sup, superficial areas of colonic tissue; TTP, time to peak; TVA, total vesselness area; vWF, von Willebrand factor; WSAVA, World Small Animal Veterinary Association

Contrast-enhanced ultrasound was performed successfully in 20/30 dogs and video clips were deemed sufficient for analysis in 16 dogs. Subjective macroscopic endoscopy scores using the WSAVA template were available for the duodenum in 29/30 dogs (median total score, 3; range, 0-16) and for the colon in 26/28 dogs (median total score, 0; range, 0-10). Duodenal inflammation was classified as lymphoplasmacytic in 11 dogs, lymphoplasmacytic and eosinophilic in 10 dogs, lymphoplasmacytic, neutrophilic and eosinophilic in 5 dogs, lymphoplasmacytic and neutrophilic in 3 dogs, and eosinophilic alone in 1 dog. Colonic biopsy specimens were classified as lymphoplasmacytic in 9 dogs, normal in 5 dogs, lymphoplasmacytic and eosinophilic in 5 dogs, lymphocytic in 3 dogs, lymphoplasmacytic, neutrophilic and eosinophilic in 2 dogs, ulcerative in 2 dogs, lymphoplasmacytic and neutrophilic in 1 dog, and eosinophilic alone in 1 dog. Mean total WSAVA histology scoring was 5 (SD = 2) for the duodenum (n = 30) and 3 (SD = 2) for the colon (n = 28).

Results of correlation analyses can be found in Figures 1 and 2. For the duodenum (Figure 1), WSAVA endoscopy scores had a moderate negative correlation (*rho* = −0.592, *P* = .004) with raw intensity density (RawID) on WL images, but with no other parameter. Duodenal WSAVA histology score showed moderate positive correlation with APV on SPIES images (*rho* = 0.424, *P* = .04), but with no other parameter. Overall, vesselness parameters for duodenal WL images showed moderate positive correlation with SPIES parameters (eg, white light total vesselness area [WLTVA] and Storz Professional Image Enhancement System total vesselness area [SPIESTVA]: *rho* = 0.454, *P* = .03; white light integrated density value [WLIDV] and Storz Professional Image Enhancement System integrated density value [SPIESIDV]: *rho* = 0.454, *P* = .03; white light area percentage value [WLAPV] and Storz Professional Image Enhancement System area percentage value [SPIESAPV]: *rho* = 0.591, *P* = .003). The white light raw intensity density (WLRawID) correlated strongly with CEUSTTP for the duodenum (*rho* = 0.814, *P* = .01), while Storz Professional Image Enhancement System raw intensity density (SPIESRawID) showed strong negative correlation with CEUS wash-in rate (*rho* = −0.714, *P* = .05). The Storz Professional Image Enhancement System area percentage value (SPIESAPV) also correlated moderately with WSAVA histology score of the duodenum (*rho* = 0.42, *P* = .04). No parameter correlated with duodenal vWF IHC results or type of histologic inflammation.

For the colon (Figure 2), WSAVA histology score correlated moderately with colonic endoscopy score (*rho* = 0.444, *P* = .02), strongly with CEUS wash-in rate (*rho* = 0.69, *P* = .006), and moderately negatively with CEUS rinse time (*rho* = −0.56, *P* = .04). White light parameters correlated strongly with SPIES parameters (*rho* = 0.687 and *P* < .001 for WLTVA and SPIESTVA, WLIDV and SPIESIDV, and

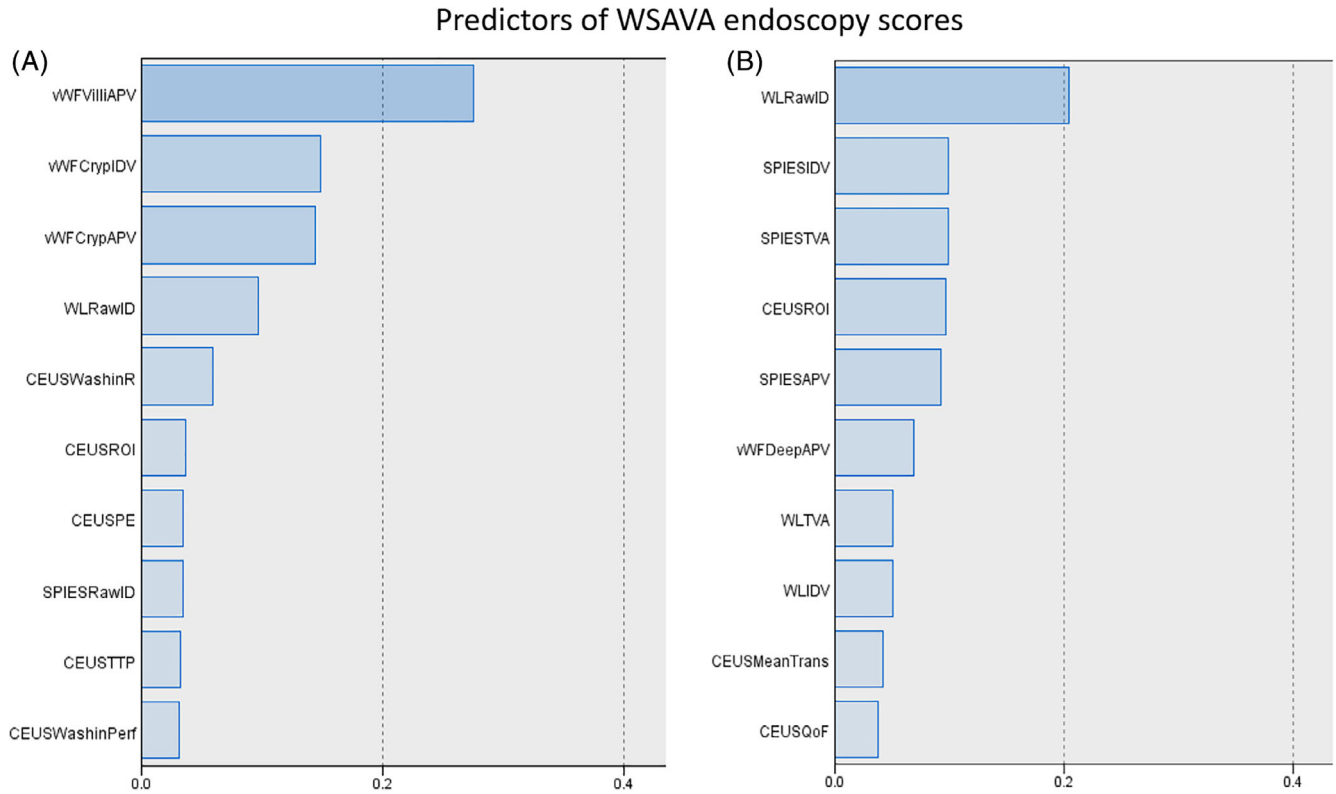

FIGURE 4 Automated stepwise linear regression modeling for factors predicting WSAVA endoscopy score of the duodenum (A) and colon (B). X-axis shows the regression coefficient. APV, area percentage value; AUC, area under the curve; CEUS, contrast-enhanced ultrasound; Col, colon; Deep, deep areas of colonic tissue; Duo, duodenum; IDV, integrated density value; Lin, linearized signal; PE, peak enhancement; Perf, perfusion index; QoF, quality of fit; RawD, raw density; RinseT, rinse time; ROI, region of interest; Sup, superficial areas of colonic tissue; TTP, time to peak; TVA, total vesselness area; vWF, von Willebrand factor; WL, white light; WSAVA, World Small Animal Veterinary Association

WLAPV and SPIESAPV, respectively). The parameter WLTVA also correlated strongly with CEUSWashinWashoutAUC (*rho* = 0.829, *P* = .04) in the colon. Colonic vWF parameters showed good to strong correlation with some of the CEUS parameters: von Willebrand factor integrated density value (vWFIDV) in superficial colonic areas correlated with CEUS rinse time (*rho* = −0.666, *P* = .009) and CEUS fall time (*rho* = −0.845, *P* = .001). In addition, CEUSTTP had a moderate negative correlation with vWFIDV (*rho* = −0.644, *P* = .01) and von Willebrand factor area percentage value (vWFAPV) (*rho* = −0.644, *P* = .01) in deep colonic areas.

For automated stepwise linear regression modeling, the WSAVA histopathology score initially was chosen as a target, because it is well established and was available for all dogs. No single parameter from CEUS, WL endoscopy, SPIES endoscopy, or vWF IHC showed potential to predominantly predict duodenal WSAVA histopathology score. Instead, several combined parameters predicted 73.4% of the model (Figure 3A). For the colonic WSAVA score, 2 parameters (vWF of the deep colonic sections and total CEUS rinse time) contributed more than others and predicted (Figure 3B) 73.1% of the model.

In a separate model, vWF expression parameters were the strongest predictors of duodenal endoscopy scores (58.1%; Figure 4A), whereas WLRawID was the predominant predictor for colonic endoscopy scores (Figure 4B), followed by SPIESIDV, SPIESTVA, Contrast-enhanced ultrasound region of interest (CEUSROI), and SPIESAPV (total model prediction quality, 72.4%). Best predictors for capillary assessment could not be established because of lack of a gold standard parameter.

4 | DISCUSSION

We assessed parameters of microvessel density and perfusion in the duodenal and colonic mucosa from dogs with CIE using novel noninvasive and minimally invasive methods, and correlated them with established parameters of mucosal inflammation. These novel methods can be integrated into routine diagnostic procedures, given the availability of the necessary equipment and some operator experience. Minimally invasive CEUS and SPIES endoscopy procedures appear feasible to assess intestinal perfusion in dogs with CIE. Endoscopy using SPIES may be a promising ancillary tool in assessing small intestinal inflammation, whereas CEUS could be used to assess colonic perfusion and inflammation. None of the assessed parameters showed convincing and consistent correlation with WSAVA histopathology scores in the duodenum, whereas CEUS rinse time was the only parameter that correlated with WSAVA histopathology scores in the colon. Although endoscopy and histology scores are established semiquantitative descriptors of inflammation in dogs with CIE that allow for comparison or correlation with results from the endoscopic scores, CEUS and vWF IHC, they are not entirely objective, and variation among pathologists persists, despite WSAVA standardization.[2-4]

Our results suggest that vesselness data acquired from both WL and SPIES endoscopy images are similar and showed moderate correlation with each other in the duodenum. One SPIES parameter (SPIESAPV) also correlated with histological scores in the duodenum. This finding may indicate that perfusion is linked to inflammation in the duodenum of these patients, and that NBI is slightly superior to WL endoscopy. However, in contrast to both the small and large intestine in humans,[40,41] vessel patterns in general were difficult to visualize in the canine intestine. Whether or not SPIES imaging endoscopy is clinically useful in this context needs further study. Previous research in duodenal inflammation of dogs assessed by CEUS, using a different product and protocol, showed correlation of some CEUS parameters and clinical signs in dogs with CIE.[16] However, no differences were detected between CIE and duodenal lymphoma or between lymphoma and control dogs, which could have been a consequence of the limited number of dogs or the nature of the control population, emphasizing the limitations of our study and the need of more robust evidence for these diagnostic tools. This need is even more important, considering that CIE and lymphoma can cause similar clinical signs and endoscopic lesions.[42]

Colonic endoscopy and histology scores correlated moderately well, which is a finding supported by other studies.[1,43,44] Similar to what was found for the duodenum, WL and SPIES parameters showed good correlation with each other. However, in contrast to the duodenum, parameters of colonic CEUS correlated inversely with vWF expression parameters as well as with type and severity of histological changes. This difference could be interpreted as indicating that faster perfusion (ie, shorter perfusion time) is caused by higher vessel density in the inflamed colonic mucosa, and should prompt further investigation of CEUS as a adjunctive tool to assess the colonic inflammation in dogs. Subjectively, the filling status of the colon did not affect or interfere with CEUS of the colonic mucosa.

Linear modeling suggests that SPIES endoscopy parameters can predict inflammation in the duodenum better than in the colon, whereas most CEUS parameters are predictors of colonic WSAVA histopathology scores. Overall, CEUS might be an attractive method for further investigations of colonic mucosal perfusion and its correlation with inflammation. When determining which parameters might influence prediction of endoscopy scores, it is of note that WL and SPIES endoscopy parameters predominated for the colon, whereas vWF IHC parameters made up the majority of the prediction model for the duodenum. This difference might reflect the fact that duodenal endoscopic lesion scoring is more difficult and might be more likely to show larger assessment variation than in the colon.[45] Also, overall WSAVA histopathology scores were lower in the colon than in the duodenum, which might have influenced our results.

Limitations of our study include the fact that clinical activity indices such as the Canine IBD activity index (CIBDAI)[46] or Canine Chronic Enteropathy Activity Index (CCECAI)[47] were not included in the analysis, because they were not routinely available. These scores could have added valuable information, because CEUS parameters can predict clinical IBD disease activity in people.[20,22] Also, our study did not attempt to include other methods to improve live endoscopy, such as chromoendoscopy, which correlates with inflammation in humans with IBD.[25] Because the use of sedation for ultrasound

examination was limited to a single agent that would not interfere with blood pressure and tissue perfusion, CEUS could only be performed in approximately 67% of the dogs. Also, because CEUS data analysis was not performed immediately after acquisition, assessing acquired images and videos for adequate quality was only possible afterward, further limiting the number of CEUS data sets available for analysis. Likewise, any prior interventions such as drug or diet trials, anesthesia, or enemas could have had an effect on the CEUS, SPIES, or endoscopy results. However, the effect of these potential cofounding factors likely is minimal. Finally, no characterization of healthy canine intestine by any of the methods employed in our study was available because of animal welfare concerns and associated ethical constraints. Unfortunately, this limitation prevents any interpretation of the data beyond the correlation analyses performed here. Establishing microvessel density and function by different means in a group of healthy dogs and comparing them to dogs with well-characterized CIE could be useful for future studies.

In conclusion, although SPIES endoscopy and CEUS might be clinically useful minimally invasive methods to assess inflammation in the duodenum and colon, respectively, they cannot substitute for histopathological assessment of mucosal biopsy specimens. Additional studies are warranted to further elucidate the relationships of the data acquired by these methods.

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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

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

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

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SUPPORTING INFORMATION

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