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# Correlating two-dimensional shear wave elastography of acute pancreatitis with Spec cPL in dogs

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## ABSTRACT

**Background:** Pancreatitis is a common disease in which 37% of dogs had evidence of acute or chronic pancreatitis at necropsy. Although biopsy is still the gold standard to diagnose acute pancreatitis, clinical data including ultrasonographic findings and measurement of canine serum pancreatic lipase immunoreactivity (cPLI) are used in routine. However, it may be insufficient in the diagnostic approach to acute pancreatitis.
**Objectives:** To evaluate the clinical diagnostic feasibility of two-dimensional shear wave elastography (2D SWE) on canine acute pancreatitis for enhanced diagnostic confidence.
**Methods:** 2D SWE was used to assess pancreatic stiffness and determine the correlation between pancreatic shear wave velocity (SWV) and Spec cPL concentration in 31 dogs with healthy pancreas and 10 dogs with acute pancreatitis.
**Results:** The pancreatic SWV was significantly higher in the acute pancreatitis group (2.67 ± 0.20 m/s) than in the healthy pancreas group (2.30 ± 0.26 m/s; $p < 0.05$). The moderate positive correlation was found between the pancreatic SWV and Spec cPL concentration (95% confidence interval, 0.214–0.693; $r = 0.489$; $p < 0.05$).
**Conclusions:** These results indicated that 2D SWE was feasible for assessing pancreatic stiffness in acute pancreatitis, and that pancreatic SWV using 2D SWE correlated with Spec cPL concentration. SWE could provide a quantitative measure of pancreatic stiffness, which can increase the accuracy of diagnosing acute pancreatitis in dogs. The 2D SWE can be used as a complementary imaging modality for diagnosing acute pancreatitis in dogs.

**Keywords:** Dog; hardness; lipase; pancreatitis; ultrasonography



## INTRODUCTION

Pancreatitis is a common disease in which 37% of dogs had evidence of acute or chronic pancreatitis at necropsy. Acute pancreatitis is sudden onset that can progress into a life-threatening disorder [1,2]. The mortality rate of acute pancreatitis is reported as 27%–58%

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**Conflict of Interest**
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in dogs [3-5]. The activation of pancreatic proteolytic enzymes results on neutrophilic inflammation of pancreas and extends to the peri-acinar fat [1]. Histopathology is the gold standard for diagnosing acute pancreatitis [6]. In acute pancreatitis, there is neutrophilic infiltrate without fibrosis or chronic inflammation that disrupts the normal architecture of the pancreas, as in the chronic pancreatitis [1]. Therefore, it can distinguish acute pancreatitis from chronic pancreatitis [7]. However, biopsy is invasive and can lead to infection, hemorrhage, and other anesthetic risks [7,8]. Therefore, instead of biopsy, ultrasonography and canine serum pancreatic lipase immunoreactivity (cPLI) are used to diagnose acute pancreatitis [9].

The cPLI measures the lipase concentration from the pancreatic acinar cells [10]. Spec cPL is an enzyme-linked immunosorbent assay that is validated in the quantitative measurement of serum concentrations of canine pancreatic lipase released by damaged pancreatic acinar cells [9,10]. Spec cPL has high sensitivity (70.0%–90.9%) and specificity (74.1%–88.0%) with low coefficient of variation (5.5%) for the diagnosis of pancreatitis [7,9,11-13]. However, Spec cPL assay is not 100% sensitive or specific, and upper gastrointestinal obstruction may increase cPL concentration [7,9]. Therefore, Spec cPL assay should not be used in isolation to make diagnosis of acute pancreatitis [7]. Besides, Spec cPL is tested in a specific laboratory with specific quality control. The turnaround time is approximately 1–2 working days, which is a distinct limitation for patients who may require immediate medical treatment and there is also additional cost and man-hour lost [11,14].

In contrast, conventional ultrasonography can evaluate the morphologic changes of the pancreas thoroughly in real-time [15]. Typical ultrasonographic findings of acute pancreatitis include the swollen pancreas with hypoechoic parenchyma indicating inflammatory change and moderately hyperechoic surrounding fats due to fat saponification [16-18]. The duodenal and/or gastric thickening, loss of intestinal motility, and regional ascites can be additionally observed [17,19]. When these typical findings are observed, the specificity of ultrasonography for acute pancreatitis is very high (92%) [20]. However, a condition of hypoalbuminemia and portal hypertension which may also show pancreatic edema can mimic acute pancreatitis [21]. In contrast, it can show normal ultrasonographic appearance in pancreatitis due to the relatively low sensitivity (68%), and the accuracy of ultrasonography is user dependent [12,15,17,20].

Although the integrated results including Spec cPL concentrations and ultrasonographic findings are commonly used in clinical diagnosis of acute pancreatitis, other non-invasive imaging modality which can increase clinical confidence in the diagnosis of acute pancreatitis is required [7,22]. Thus, there are previous studies about diagnosing acute pancreatitis using advanced imaging modality such as contrast-enhanced ultrasonography, and computed tomography angiography [22,23]. However, there is yet no specific recommended imaging modality or standards for the diagnosis of canine acute pancreatitis [7].

In pancreatitis, inflammatory and edematous parenchyma can make pancreatic tissue stiffened [5]. However, conventional ultrasonography cannot assess the mechanical properties of the lesion [24]. Shear wave elastography (SWE) is an emerging technique that evaluates the tissue stiffness and estimated the pathologic change of the tissue [25]. In contrast to computed tomography angiography and contrast-enhanced ultrasonography, which require the administration of the contrast medium or anesthetics, SWE is easily combined with conventional ultrasonography [25]. Moreover, in humans, the typical

sonographic features of acute pancreatitis can be seen in a small number of patients, and the acute change of the pancreatic tissue can show various appearances in conventional ultrasonography. Therefore, SWE application to the pancreas has been actively studied in human medicine [25-28]. The studies demonstrated the usefulness of SWE in characterizing and differentiating normal pancreas and acute pancreatitis [25-28]. Meanwhile, in veterinary medicine, only one study evaluated the SWE application in pancreatic disorders and demonstrated the higher pancreatic shear wave velocity (SWV) in pancreatitis compared to healthy pancreas [29]. However, the study assessed the SWV changes in pancreatitis including acute and chronic forms without discrimination of the disease progression [29]. Moreover, considering the comprehensive diagnosis of the acute pancreatitis using clinical signs, laboratory test, and ultrasonography, it is needed that the correlation of the SWV of the pancreas with Spec cPL concentration which is more sensitive than conventional ultrasonography in the diagnosis of acute pancreatitis.

In this study, we performed two-dimensional (2D) SWE in dogs with healthy pancreas and acute pancreatitis and evaluated the correlation between the pancreatic SWV and Spec cPL concentration of dogs with acute pancreatitis. We hypothesized that pancreatic SWV of acute pancreatitis would be significantly higher than that of healthy pancreas due to its acquired tissue changes and have a positive correlation with Spec cPL concentration. The aims of this study were to evaluate the feasibility of 2D SWE for assessing acute pancreatitis, as well as to corelate the correlation between pancreatic SWV and serum pancreatic lipase concentration using Spec cPL in dogs.

## MATERIALS AND METHODS

This clinical study included client-owned dogs that underwent 2D SWE of the pancreas at Doctor Dog Animal Medical Center from January 2021 to July 2021. The dogs were included in this study: 1) when conventional ultrasonography, 2D SWE, and laboratory tests including Spec cPL were performed, and 2) when reliable 2D SWE results were obtained. The dogs were excluded from this study: 1) when other conditions that can affect pancreatic stiffness were found, such as large amounts of peritoneal effusion, 2) when a pancreatic mass lesion such as an adenocarcinoma or insulinoma was found, and 3) when the pancreatic inflammation was caused by diffuse abdominal inflammation such as peritonitis or inflammatory bowel disease. The medical record was reviewed to obtain the age, sex, breed, and body weight in all dogs. The study protocol was approved by the Institutional Animal Care and Use Committee at Chonnam National University (CNU IACUC-YB-2021-54).

Dogs were divided into two groups including the healthy pancreas group and acute pancreatitis group based on history, laboratory tests including Spec cPL, and conventional ultrasonography. Dogs in the healthy pancreas group were selected using the following criteria: 1) no clinical signs associated with pancreatic disease such as anorexia, epigastric pain, vomiting, and diarrhea; 2) Spec cPL concentration ≤ 200 μg/L; and 3) normal appearance of the pancreas on conventional ultrasonography. The acute pancreatitis group was selected using the following criteria: 1) the presence of clinical signs associated with pancreatic disease, 2) Spec CPL concentration ≥ 400 μg/L, and 3) ultrasonographic findings of acute pancreatitis including pancreatic enlargement, hypoechoic regions within the pancreas, increased echogenicity of the surrounding mesentery, and altered pancreatic echotexture [20].

All dogs underwent conventional ultrasonography and 2D SWE, and these two examinations were performed using the same ultrasound machine (EPIQ 5; Philips, USA) with a 4–18 MHz linear array transducer. The conventional ultrasonography and 2D SWE were performed by one veterinarian with six years of radiology experience (H.C.). Ultrasonography and 2D SWE were not performed in a blind manner due to the epigastric pain and typical conventional ultrasonographic images of acute pancreatitis. All dogs were manually restrained without sedation or anesthesia.

After clipping the abdominal hair and applying the adequate gel to the area of examination, conventional ultrasonography of the entire parenchyma of the pancreas was performed from the pancreatic body, right lobe, and left lobe in this order. The size, echogenicity, and contour of the pancreas and peri-pancreatic areas were evaluated.

After conventional ultrasonography, 2D SWE of the right pancreatic lobe was performed using the installed software (ElastQ Imaging; Philips) according to the guideline of the manufacturer and previous studies [29-33]. After placing the dog in left lateral recumbency with extension of the four limbs, the linear probe was placed on the right cranial abdomen perpendicular to the abdominal surface, with care taken to apply minimal compression, and the ultrasonographic image of the right pancreatic lobe was achieved using the subcostal approach.

The 2D SWE was launched at end-expiration while the dogs were breathing regularly. On dual screen mode, the color-coded map and B-mode image were displayed side by side. In the color-coded map, a rectangular, color-coded elastographic box was placed over the 2D images. Blue color represented low SWV, and red represented high SWV in the color-coded map. A confidence map was also simultaneously generated within the box (**Fig. 1**). The areas with a confidence value of less than 50% are shown as color defects in the color-coded map [34].

The frame that showed the consistent color-coded elastographic box was chosen, and a round region of interest (ROI) of 3 mm in diameter was placed at 5 to 20 mm from the skin surface within the color-coded elastographic box while avoiding the rib shadows or areas near blood vessels. The beam focus was placed at the level of the ROI. In each ROI, the pancreatic SWV

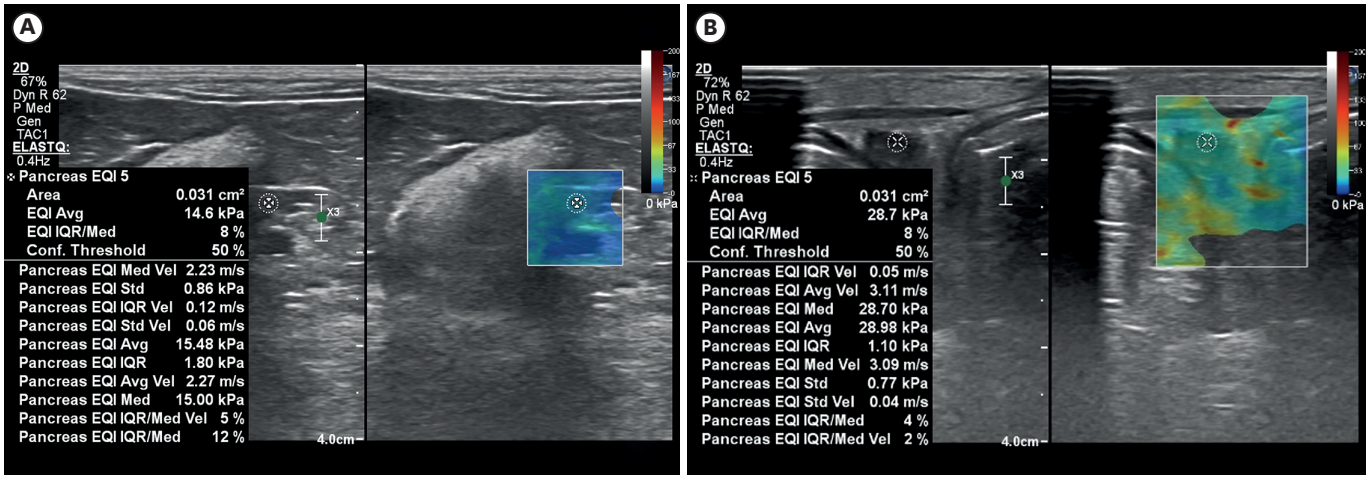


**Fig. 1.** Elastographic images of the pancreas using two-dimensional shear wave elastography. B-mode image (left) and color-coded map (right) and are displayed. A region of interest is located at the right limb of pancreas in a dog with healthy pancreas (A) and a dog with acute pancreatitis (B). Pancreatic shear wave velocity results are described in the left corner of each image.


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was recorded in meters/seconds (m/s), and the interquartile range to median values (IQR/MED) was also automatically calculated.

The IQR/MED was used to assess the quality of the measurement by evaluating the variability of the data. The data in the ROI was determined as valid when IQR/MED was less than 30%. In each dog, the measurement was performed until five valid data was achieved, and the median value of the five valid data was recorded as the representative pancreatic SWV in the dog. If the IQR/MED of the five valid data was over 30%, it was determined as an unreliable measurement and excluded from further analysis.

Blood sampling for Spec cPL was conducted on the same day of ultrasonography and 2D SWE. Approximately 3 mL of whole blood samples were collected in lithium heparin tubes (Vacuet; Greiner Bio-One, Germany) and incubated at room temperature until it formed clots. Serums isolated using centrifugation (7 min, 3,500 rpm) were transported to a commercial laboratory, and the Spec cPL assay was performed (IDEXX, USA).

Statistical analysis was performed using a statistical program (IBM SPSS Statistics ver. 25, IBM Corporation, USA) under the supervision of one statistician (J.K.K.). Normal distribution of the data was tested using Kolmogorov-Smirnov test. Then, Pearson correlation coefficient was used to analyze the correlations of pancreatic SWV with signalment (age, body weight), and Spec cPL concentration. The difference in pancreatic SWV between the healthy pancreas group and acute pancreatitis group was evaluated by Student t-test. The sensitivity, specificity, and the area under the curve (AUC) were assessed using receiver operating characteristics (ROC) curve analysis. The cut-off value for predicting acute pancreatitis was chosen to maximize the specificity and sensitivity. Data showing normal distribution are presented as mean ± SD. The level of significance was set at $p < 0.05$.

## RESULTS

A total of 42 dogs that underwent ultrasonography and 2D SWE were selected in this study. All SWE examinations were performed without any severe complications such as vomiting or persistent pain on the examined area. However, one dog in the healthy pancreas group, whose body weight was approximately 3 kg, was excluded from the analysis because its unreliable measurement based on over 30% of IQR/MED of the five valid data. Therefore, 41 dogs were finally enrolled in this study. The breed, sex, age, and bodyweight of the dogs in the healthy pancreas group and acute pancreatitis group are presented in **Table 1**.

**Table 1.** Pancreatic SWV, breed, gender, age, and bodyweight of healthy pancreas group and acute pancreatitis group

| Variables | Healthy pancreas group (n = 31) | Acute pancreatitis group (n = 10) |
|---|---|---|
| Pancreatic SWV (m/s) | 2.30 ± 0.26 | 2.67 ± 0.20 |
| Breed (n) | Maltese (6), Pomeranian (5), Shih Tzu (4), Yorkshire Terrier (4), Dachshund (3), Mixed (3), Poodle (2), Boston Terrier (1), Bull Terrier (1), Chihuahua (1), Japanese Spitz (1) | Poodle (4), Maltese (3), Shih Tzu (2), Yorkshire Terrier (1) |
| Sex (n) | Neutered male (15), neutered female (12), intact male (1), intact female (3) | Neutered male (6), neutered female (2), intact male (1), intact female (1) |
| Age (yr) | 9.16 ± 4.02 | 10.44 ± 3.83 |
| Body weight (kg) | 5.35 ± 3.28 | 4.79 ± 1.45 |

Data are presented as mean ± SD.
SWV, shear wave velocity.


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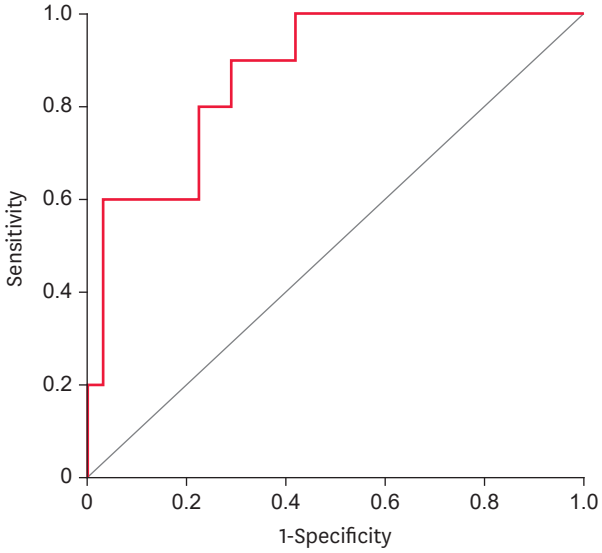


**Fig. 2.** ROC curve for pancreatic SWV in distinguishing between healthy pancreas group and acute pancreatitis group. Area under the curve for pancreatic SWV was 0.871. The cut-off value of ROC curve is 2.43 m/s with a 71.0% sensitivity and 90.0% specificity. The solid line represents reference line.
ROC, receiver operating characteristics; SWV, shear wave velocity.

The pancreas was visualized as a homogenous blue-to-green color mapping in the healthy pancreas group on 2D SWE. In acute pancreatitis, some areas of the pancreas were visualized as green-to-yellow color mapping. The pancreatic SWV did not show a significant correlation with body weight in the healthy pancreas group ($p = 0.135$) and acute pancreatitis group ($p = 0.105$). In addition, there was no significant correlation between pancreatic SWV and age in the healthy pancreas group ($p = 0.785$) and acute pancreatitis group ($p = 0.374$).

The pancreatic SWV of the healthy pancreas group and acute pancreatitis group showed normal distribution, and the pancreatic SWV was significantly higher in the acute pancreatitis group compared with the healthy pancreas group (**Table 1**). The AUC of the ROC curve of pancreatic SWV between the healthy pancreas group and acute pancreatitis group was 0.871 with a 95% confidence interval of 0.756 to 0.986 ($p < 0.05$; **Fig. 2**). The cut-off value of pancreatic SWV for the acute pancreatitis group was set as 2.43 m/s with a 71.0% sensitivity and 90.0% specificity. The Spec cPL concentration was measured as 68.00 ± 47.83 μg/L in the healthy pancreas group and 1,081.40 ± 729.60 μg/L in the acute pancreatitis group. The moderate positive correlation was found between the pancreatic SWV and Spec cPL concentration ($r = 0.489$; $p < 0.05$) (**Fig. 3**).

## DISCUSSION

The 2D SWE was applied to dogs with healthy pancreas and acute pancreatitis, and the pancreatic SWV was successfully measured without sedation or anesthesia. The 2D SWE was feasible for assessing pancreatic stiffness, having a correlation with Spec cPL concentrations in dogs.

2D SWE performed the qualitative evaluation of pancreatic stiffness from the right pancreatic lobe on the color-coded map based on whether the color was homogenous and heterogenous

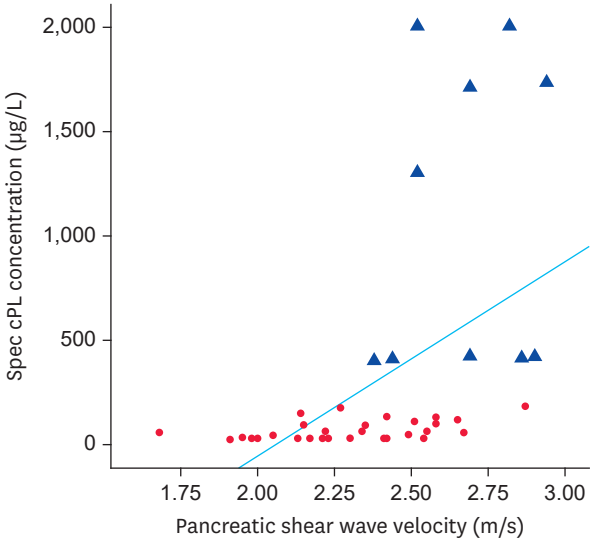


**Fig. 3.** Correlation analysis of Spec cPL concentration and pancreatic SWV. There is the moderate positive correlation between the pancreatic SWV and Spec cPL concentrations ($r$ = 0.489; $p$ = 0.001). Circle and triangle denote the healthy pancreas group and acute pancreas group, respectively. Solid line represents regression line. SWV, shear wave velocity.

and major color present [32]. In normal dogs, the pancreas was displayed in blue to green color, whereas in dogs with acute pancreatitis, the pancreas was displayed in green in the most parts, and green to yellow in minor parts. This appearance was compatible to the previous report, which revealed that healthy canine pancreas showed a homogenous bluish color, whereas pancreatitis showed some areas of yellowish color [29]. In human medicine, the color patterns of pancreatic elastography reveal that healthy pancreas shows a homogeneous green pattern, and benign inflammation of the pancreas appears as a heterogeneous mixed-colored pattern, honeycomb predominantly blue pattern, or a homogeneously green pattern [35]. Although this color pattern was easy to understand, the judgement could be subjective [31].

2D SWE provided quantitative data about the pancreatic SWV from the right pancreatic lobe in this study, and the acute pancreatitis had significantly higher pancreatic SWV than healthy pancreas. This result was compatible with the previous study which revealed that the pancreas with pancreatic diseases was stiffer compared to healthy pancreas in dogs [29]. However, the previous study assessed the changes in pancreatic stiffness including acute and chronic pancreatitis regardless of the progress of the pancreatic inflammation; therefore, the acute changes in pancreatic SWV could not be determined separately. In human studies, acute pancreatitis showed the increase in stiffness of the pancreatic parenchyma compared to healthy pancreas [25,27,36].

There is also a possibility that the pancreatic SWV of dogs reflect different histopathologic change of the pancreas in various diseases. In a previous study, the pancreatic SWV was significantly different between inflammation (acute and chronic pancreatitis) and pancreatic adenocarcinoma [37]. In this study, the increase of pancreatic stiffness was considered to be closely related to histologic changes including the neutrophilic inflammation, driven by the cytokine, complement, and kinin systems within the pancreatic parenchyma and edematous parenchyma, and expansion of the pancreatic interstitium by lightly proteinaceous fluid in acute pancreatitis [2,38]. However, we could not completely rule out the effect of technical

factor on the pancreatic SWV, because most dogs with acute pancreatitis had epigastric pain and increased abdominal pressure.

In this study, we assessed the clinical relevance of the pancreatic SWV by comparing it with Spec cPL concentration because Spec cPL is considered as the most sensitive method to determine acute pancreatitis in the clinic. There was the moderate positive correlation between the pancreatic SWV and Spec cPL concentrations in dogs. This result could not be compared with that of other studies because the comparison between pancreatic stiffness and Spec cPL concentration has never been attempted before. A previous human study revealed that there was no significant correlation between the pancreatic stiffness and amylase level [39]. Meanwhile, in another study, the Spec cPL concentration was correlated with the pancreatic inflammation score estimated by histopathologic examination; however, there was no correlation between the Spec cPL concentration and the fibrosis score, commonly used in diagnosing chronic pancreatitis [38]. Hence, the increased pancreatic SWV and Spec cPL concentration observed in this study may be related to the inflammatory changes associated with acute pancreatitis.

There were several limitations in this study. First, the number of dogs with acute pancreatitis was relatively small because only patients that underwent Spec cPL testing, conventional ultrasonography, and 2D SWE were included in this study. Second, the 2D SWE was performed only from the right pancreatic lobe, but not from the pancreatic body and left pancreatic lobe. We chose to perform 2D SWE at the right pancreatic lobe study because the right pancreatic lobe tends to be the most commonly affected in canine pancreatitis, and reproducibility of 2D SWE at the right pancreatic lobe is superior to that of other lobes [30,40]. In contrast, the pancreatic left lobe lies behind the greater curvature of the stomach and adjacent to the cranial aspect of the transverse colon, and pancreatic body lies adjacent to the left limb [40]. In this study, focal lesion such as pancreatic pseudocyst or abscess secondary to the acute pancreatitis was not detected on conventional ultrasonography. In further studies, 2D SWE of the left pancreatic lobe and pancreatic body needs to be performed in dogs. In addition, the pancreatic region for 2D SWE can be selected after initially observing the pancreas using conventional ultrasonography. Third, the healthy pancreas and acute pancreatitis were not confirmed through histologic examination of the pancreas. Instead, we performed Spec cPL and conventional ultrasonography and assessed the history and clinical signs of the dogs. Fourth, majority of dogs enrolled in this study were small breed dogs. Thus, the effect of body size on pancreatic SWV could not be evaluated.

In conclusion, there was a positive correlation between 2D SWE and stiffness of the pancreas undergoing acute inflammation. These results indicated that 2D SWE is feasible for assessing pancreatic stiffness in acute pancreatitis. Further study is needed for assessing the potential of 2D SWE for scoring the severity of acute pancreatitis or treatment regimes by the quantitative measure of pancreatic stiffness in dogs.

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