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Quantitative analysis of contrast-enhanced ultrasound estimates intrahepatic portal vascularity in dogs with single extrahepatic portosystemic shunt

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OBJECTIVE
To evaluate the clinical impact on quantitative analysis of contrast-enhanced ultrasound (CEUS) on single extrahepatic portosystemic shunt (PSS) in dogs.

ANIMALS
21 client-owned dogs with single extrahepatic PSS and 5 healthy Beagles.

PROCEDURES
In all dogs, CEUS was performed to calculate the rising time (RT), rising rate (RR), and portal vein-to-hepatic parenchyma transit time (ΔHP-PV) from the time-intensity curve obtained in the hepatic parenchyma and portal vein. All dogs in the PSS group underwent preoperative CT angiography (CTA) and surgery. The CEUS variables in the PSS group were compared with those in the healthy dogs (control group) and were analyzed for shunt types and grades of intrahepatic portal venous branches based on CTA findings, intraoperative portal pressure, and surgical procedures.

RESULTS
All 3 CEUS variables showed no significant differences between the PSS and control groups. The RT and ΔHP-PV in the left gastrophrenic shunt group were significantly longer than in the other shunt types. In the intrahepatic portal vascularity, the RT in grade 1 was significantly shorter than in grades 3 and 4, and the RR in grade 1 was significantly higher than in grade 4. The RT and ΔHP-PV were significantly correlated with portal pressure variables. The RT in dogs with partial ligation was significantly shorter than in dogs with complete ligation and percutaneous transvenous coil embolization.

CLINICAL RELEVANCE
Quantitative assessments of CEUS may be useful for estimating intrahepatic portal vascularity in dogs with single extrahepatic PSS.

Congenital portosystemic shunt (PSS) involves anomalous vessels that allow normal portal blood to pass directly into the systemic circulation[1] and is categorized into extrahepatic and intrahepatic types based on the shunt location. Intrahepatic PSS tends to occur in large-breed dogs, whereas extrahepatic PSS is more prevalent in small-breed dogs.[1] Traditionally, extrahepatic PSS was classified simply as portocaval or portoazygos. However, recently CT angiography (CTA) has described detailed shunt morphology.[2–5] The major shunt types include left gastrophrenic shunt (LGP), splenocaval shunt, left gastrocaval shunt (LGC), right gastrocaval shunt (RGC), and left gastroazygos shunt and right gastroazygos shunt. The differences of shunt types affect the age at diagnosis, frequency of clinical signs, alkaline phosphatase activities, fasting ammonia concentration, portal vein/aortic ratio, shunt fraction (SF), hepatic arterial blood flow, and portal venous blood flow.[3,4,6]

Surgery is the treatment of choice for PSS,[1,7] and surgical attenuation, ameroid constrictor placement (ACP), cellophane banding, and percutaneous transvenous coil embolization (PTCE) are often chosen.[8–12] The degree of shunt attenuation is determined using CTA, operative findings, intraoperative portal venous pressure, and portovenography.[1] The acute occlusion of the shunt vessel can lead to excessively increased portal pressure and may result in multiple acquired shunts or death related with portal hypertension. Thus, the intraoperative measurement of portal venous blood pressure is performed as a guide for the degree of attenuation for animals undergoing complete ligation (CL) or partial ligation (PL).[1]

A previous report[13] demonstrated that assessing intrahepatic portal venous branches in portovenography could help determine the degree of shunt attenuation and may predict clinical outcomes postoperatively. In one study,[14] the absence of arborizing intrahepatic vasculature on preligation portovenography was correlated with a greater incidence of postoperative complications. Therefore, the preoperative evaluation of shunt type, portal pressure, and intrahepatic portal branch development is clinically important. However, CTA requires anesthesia, and portal pressure measurement and portal angiography are invasive, requiring laparotomy. Therefore, less invasive, simpler techniques are desirable for assessing PSS in dogs.

The first report[15] on contrast-enhanced ultrasound (CEUS) for canine PSS evaluated 3 dogs with extrahepatic PSS using the contrast agent perflutren lipid microsphere in 2003. According to this report, the time-intensity curve (TIC) in canine PSS showed that the time to peak was shorter and the inflow slope was steeper than normal dogs. The second report[16] on CEUS for canine PSS evaluated 9 dogs with extrahepatic PSS using the contrast agent perflubutane in 2019 and showed that quantitative assessments had 100% sensitivity and 75.0% to 87.5% specificity in the diagnosis of PSS. These reports have considered that CEUS with PSS reflected a compensatory increase in the hepatic arterial blood flow in the liver to decrease intrahepatic portal blood flow. Therefore, CEUS may reflect hepatic circulation and be correlated with hepatic portal blood volume and portal pressure.

Dogs with PSS had a large range of SF.[6] Thus, hepatic portal and shunt blood volumes depend on cases. However, past reports in CEUS for canine PSS involved few cases and were insufficient to reveal a relationship between quantitative assessments and intrahepatic portal blood volume and pressure.

We hypothesized that shunt types, surgical treatments, intrahepatic portal vein branch grades, and portal venous pressure would have an effect on quantitative assessments of dogs with PSS in CEUS. Therefore, the purpose of this study was to evaluate quantitative assessments of CEUS regarding shunt types, surgical treatments, intrahepatic portal venous branch grades, and portal venous pressure.

Materials and Methods

Study design

This was a prospective case study. All dogs with PSS (PSS group) were referred to our hospital with suspected PSS between August 2019 and February 2021. Informed consent was obtained prior to the inclusion of dogs in the study. The dogs were evaluated by physical examinations, blood and urine tests, radiography, ultrasonography, and CTA in our hospital. A definitive diagnosis of PSS was made using CTA in our hospital. The inclusion criteria of this study were dogs with extrahepatic PSS showing contrast enhancement by CEUS. After CEUS, all the recruited dogs underwent surgery.

In the control group, 5 healthy Beagles (5 males, 2.8 to 3.3 years, and 8.0 to 10.9 kg) were included. All dogs were confirmed as healthy based on physical examination, blood examination, and abdominal ultrasound. This study was approved by the Nihon University Animal Care and Use Committee (AP20BRS020-1).

CEUS

All dogs underwent CEUS as follows: a suspension of perflubutane (Sonazoid; Daiichi-Sankyo Corp) was prepared with 2 ml of saline at a concentration of 8 µl/ml. An ultrasound machine (Aplio 400; Canon Medical Systems Corp) was used with a 6-MHz convex probe for CEUS. In the PSS group, a 22- or 24-gauge intravenous catheter was inserted into the cephalic vein depending on the animal's size. In the control group, a 22-gauge intravenous catheter was inserted into the cephalic vein. The contrast medium was injected intravenously at a volume of 0.015 ml/kg (perflubutane, 0.12 µl/kg), followed by a 5-ml saline injection. All dogs were positioned in the left lateral recumbency position, and right divisions in the liver and portal vein were shown in the right intercostal approach. This view was recorded for 30 s after the saline flush in the contrast harmonic imaging mode. The mechanical index was set under 0.2. Contrast-enhanced ultrasound was performed by a veterinarian, and PSS dogs underwent CEUS on the day before the operation. None of the dogs was sedated or anesthetized.

CEUS image analysis

All images were analyzed by a veterinarian. Region of interests (ROIs) were placed on the hepatic parenchyma (HP) and portal vein, and TICs were generated. The ROI of HP in PSS dogs was 5 mm in diameter and positioned 1.5 to 3 cm in depth. The ROI of the HP in the control group was 5 mm in diameter and positioned 3 cm in depth. The ROI size of the portal vein was 1 to 5 mm, depending on the vein size. The ROI position was corrected to minimize body movement and respiration (ROI tracking). Rising time (RT), rising rate (RR), and portal vein-to hepatic parenchyma transit time (ΔHP-PV) were calculated from the TICs in the control and PSS groups. The RT was defined as the interval between the initial rise and attainment of its maximum signal intensity. RR was defined as the slope of the initial rise and attainment of its maximum signal intensity. ΔHP-PV was defined as the transit time of the contrast medium from the portal vein to the HP.

CTA findings

The shunt types and grades of the intrahepatic portal venous branches were determined using CTA. This grading system reported in portovenography[13] was applied to CTA in this study and defined as follows: grade 1, no intrahepatic portal vasculature visible; grade 2, faint opacification of vestigial portal vessels; grade 3, faint opacification of a few second- or third-generation portal vessels; and grade 4, substantial opacification of third- and fourth-generation portal vessels. In addition, the grades of intrahepatic portal venous branches were categorized in two groups; grades 1 and 2 were in the low-grade group and grades 3 and 4 were in the high-grade group.

Intraoperative findings

All dogs underwent the surgical procedure by 1 veterinarian, who decided either CL, PTCE, ACP, or PL. The portal venous blood pressure was measured intraoperatively before and during the temporary occlusion of the shunt vessel. The ratio of increase in portal venous blood pressure by temporary occlusion of the shunt vessel was calculated in portal venous blood pressure during temporary occlusion of the shunt vessel/portal venous blood pressure before temporary occlusion of the shunt vessel.

Data analysis

Statistical tests were performed using commercially available statistical analysis software (GraphPad Prism version 6.0 for Macintosh; GraphPad Software Inc). The RT, RR, and ΔHP-PV were statistically compared between the control and PSS groups and between the low- and high-grade groups of intrahepatic portal venous branches using the Mann-Whitney test. Between the shunt types and control group, and surgical treatments and control group, intrahepatic portal venous branch grades were statistically compared using the Kruskal-Wallis test followed by Dunn's *post hoc* correction test. The correlations between these parameters and portal venous pressures before and during temporary occlusion and the ratio of increase in portal venous blood pressure by temporary occlusion of the shunt vessel were evaluated by assessing the Spearman rank correlation coefficient. Differences were considered statistically significant at $P < 0.05$.

Results

Animals

Twenty-one client-owned dogs met the inclusion criteria and were enrolled in this study. The median age of dogs included in this study was 1.8 years (range, 0.3 to 11.7 years). The 21 dogs included 6 (2 castrated) males and 15 (6 spayed) females. The median body weight of the dogs was 4.0 kg (range, 1.5 to 14.4 kg). The breeds were as follows: 4 Papillons; 3 mixed breeds; 2 each Miniature Dachshunds, Toy Poodles, and Miniature Schnauzers; and 1 each Pomeranian, Yorkshire Terrier, Pekingese, Maltese, Pug, Shih Tzu, Jack Russell Terrier, and Chihuahua.

CTA findings

Of the 21 dogs, 11 (52.4%) had LGP and 10 (47.6%) had the other shunt types, including 3 LGCs, 3 RGCs, 2 left gastroazygos shunts, a right-left gastrocaval shunt, and a splenophrenicoabdominal shunt. Of the intrahepatic portal venous branches, 4 had grade 1, 3 had grade 2, 5 had grade 3, and 9 had grade 4.

Intraoperative findings

Intraoperative portal venous blood pressure was measured in 21 dogs. The median portal venous pressures before and during temporary occlusion were 5 (range, 1 to 13 mm Hg) and 10 mm Hg (range, 5 to 46 mm Hg), respectively. The median ratio of increase in portal venous blood pressure by temporary occlusion of the shunt vessel was 2.5-fold (range, 1.0-fold to 12.5-fold). Of the 21 dogs that underwent surgical treatment, 8 (38.1%) had CL, 5 (23.8%) had PTCE, 4 (19.0%) had ACP, and 4 (19.0%) had PL.

CEUS findings

Of the 21 dogs, HP and portal vein were described in 17 dogs, and only HP was described in 4 dogs, in which the portal vein was insufficiently visible due to the interference of gas in the stomach and duodenum. The details are listed **(Supplementary Table S1)**. Between the control and PSS groups, RT ($P = 0.899$), RR ($P = 0.912$), and ΔHP-PV ($P = 0.446$) did not differ significantly **(Supplementary Table S2)**.

The parameters of the shunt types are shown **(Figure 1 and Table 1)**. The RR did not differ significantly between the groups ($P = 0.142$). However, the RT ($P = 0.019$) and ΔHP-PV ($P = 0.041$) in the

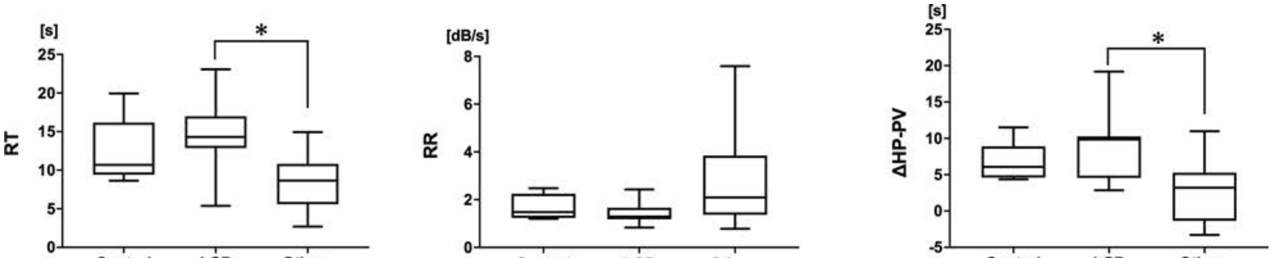

Figure 1—Box-and-whisker plots for the results of quantitative assessments in contrast-enhanced ultrasound stratified by left gastrophrenic shunt (LGP), other shunt types and control group provided to 21 dogs with portosystemic shunt and 5 normal dogs. Each box represents results for the 25th to 75th percentiles, the horizontal line in the box represents the median of each parameter value, and the whiskers represent the range of each parameter value. Others mean the other shunt types including 3 left gastrocaval shunts, 3 right gastrocaval shunts, 2 left gastroazygos shunts, a right-left gastrocaval shunt, and a splenophrenicoabdominal shunt. dB/s = Decibel per second. ΔHP-PV = Portal vein-to-hepatic parenchyma transit time. RR = Rising rate. RT = Rising time. *$P < 0.05$ by Dunn's *post hoc* correction test.

Table 1—Median [Range] rising rate (RT), rising rate (RR), and ΔHP-PV of contrast-enhanced ultrasound (CEUS) in 5 normal dogs (Control), 11 dogs with left gastrophrenic shunt (LGP), and 10 dogs with other shunt types.

	RT (s)		RR (dB/s)		ΔHP-PV (s)	
Variable	**Median [Range]**	***n***	**Median [Range]**	***n***	**Median [Range]**	***n***
Control	10.68 [8.63–19.94]	5	1.48 [1.20–2.48]	5	6.06 [4.35–11.49]	5
LGP	14.30[a] [5.38–23.06]	11	1.29 [0.83–2.43]	11	9.84[a] [2.86–19.19]	8
Other shunt types	8.65 [2.69–14.93]	10	2.09 [0.78–7.59]	10	3.20 [−3.30–10.98]	9

dB/s = Decibel per second.
[a]Significantly higher than other shunt types.
Other shunt types include 3 left gastrocaval shunts, 3 right gastrocaval shunts, 2 left gastroazygos shunts, a right-left gastrocaval shunt, and a splenophrenicoabdominal shunt.

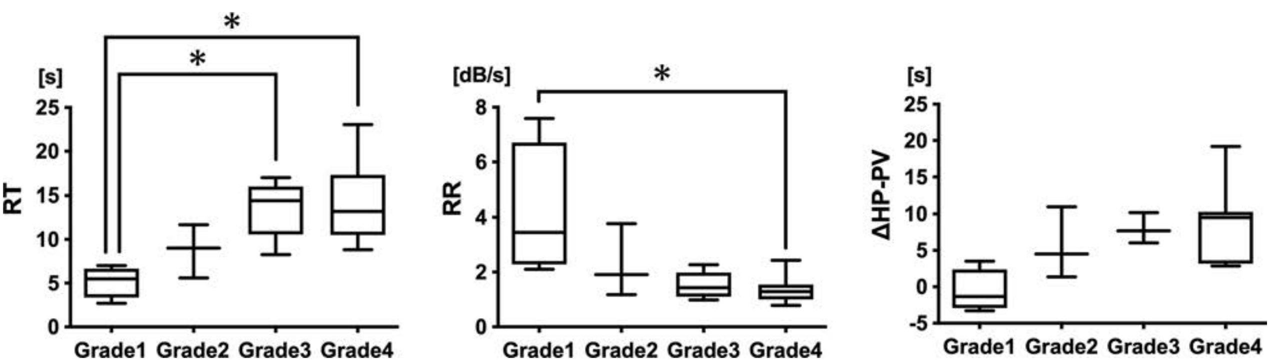

Figure 2—Box-and-whisker plots for the results of quantitative assessments in contrast-enhanced ultrasound stratified by the grades of the intrahepatic portal vein branches assigned to 21 dogs with portosystemic shunt. Each box represents results for the 25th to 75th percentiles, the horizontal line in the box represents the median of each parameter value, and the whiskers represent the range of each parameter value. dB/s = Decibel per second. *$P < 0.05$ by Dunn's *post hoc* correction test.

Table 2—Median [Range] RT, RR, and ΔHP-PV of CEUS in 21 portosystemic shunt (PSS) dogs with each grade of intrahepatic portal venous branch.

	RT (s)		RR (dB/s)		ΔHP-PV (s)	
Variable	**Median [Range]**	***n***	**Median [Range]**	***n***	**Median [Range]**	***n***
Grade 1	5.48[a] [2.69–7.01]	4	3.45[b] [2.10–7.59]	4	−1.34 [−3.30–3.51]	4
Grade 2	9.02 [5.60–11.67]	3	1.91 [1.18–3.77]	3	4.51 [1.36–10.98]	3
Grade 3	14.42 [8.27–16.99]	5	1.43 [0.98–2.27]	5	7.69 [6.03–10.18]	3
Grade 4	13.20 [8.82–23.06]	9	1.29 [0.78–2.43]	9	9.51 [2.86–19.19]	7

dB/s = Decibel per second.
[a]Significantly lower than grades 3 and 4.
[b]Significantly higher than grade 4.

LGP group were significantly longer than in the other shunt types. These parameters in the intrahepatic portal venous branch grades are shown **(Figure 2 and Table 2)**. In grade 1, the RT was significantly shorter than in grades 3 ($P = 0.041$) and 4 ($P = 0.014$). The RR in grade 1 was significantly higher than that in grade 4 ($P = 0.025$). The ΔHP-PV did not differ significantly between the intrahepatic portal venous branch grades ($P = 0.081$). The parameters of the low- and high-grade groups are shown **(Supplementary Table S3)**. The RT in the low-grade group was significantly shorter than that in the high-grade group ($P = 0.001$). The RR in the low-grade group was significantly higher than that in the high-grade group ($P = 0.008$). On the other hand, ΔHP-PV did not have a significant difference between the low- and high-grade groups ($P = 0.055$).

The RT ($P = 0.857$; $r = −0.042$), RR ($P = 0.859$; $r = 0.041$), and ΔHP-PV ($P = 0.658$; $r = −0.106$) did not correlate significantly with portal venous pressure before temporary occlusion. In portal venous pressure during temporary occlusion, RT ($P = 0.019$; $r = −0.509$) and ΔHP-PV ($P = 0.026$; $r = −0.537$) were significantly negatively correlated. On the other hand, RR did not correlate with portal venous pressure during temporary occlusion ($P = 0.228$, $r = 0.275$). In the ratio of increase in portal venous blood pressure by temporary occlusion of the shunt vessel, RT ($P = 0.007$; $r = −0.574$) and ΔHP-PV ($P = 0.021$; $r = −0.558$) had a significant negative correlation, and RR ($P = 0.033$; $r = 0.468$) was significantly weakly positively correlated.

These parameters in the surgical treatments are shown **(Figure 3 and Table 3)**. The RT in the PL

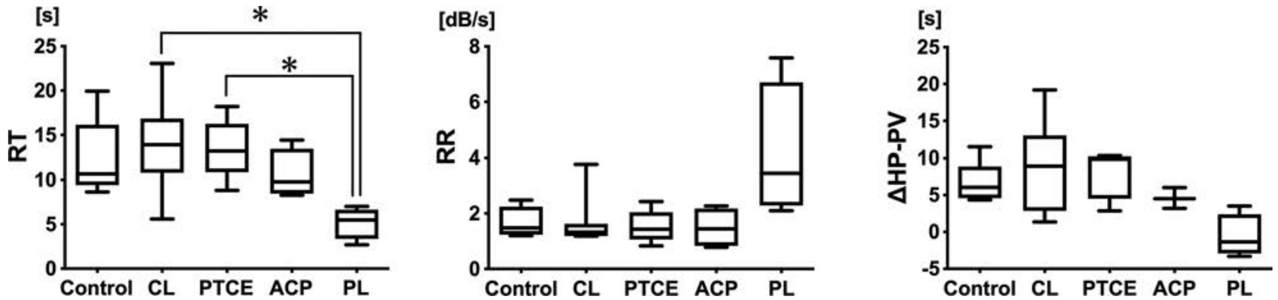

Figure 3—Box-and-whisker plots for results of quantitative assessments in contrast-enhanced ultrasound stratified by surgical treatments and control group provided to 21 dogs with portosystemic shunt and 5 normal dogs. Each box represents results for the 25th to 75th percentiles, the horizontal line in the box represents the median of each parameter value, and the whiskers represent the range of each parameter value. ACP = ameroid constrictor placement. CL = Complete ligation. dB/s = Decibel per second. PL = partial ligation. PTCE = Percutaneous transvenous coil embolization. *$P < 0.05$ by Dunn's *post hoc* correction test.

Table 3—Median [Range] RT, RR, and ΔHP-PV of CEUS in 5 normal dogs (Control) and 21 PSS dogs with each surgical treatment.

	RT (s)		RR (dB/s)		ΔHP-PV (s)	
Variable	**Median [Range]**	***n***	**Median [Range]**	***n***	**Median [Range]**	***n***
Control	10.68 [8.63–19.94]	5	1.48 [1.20–2.48]	5	6.06 [4.35–11.49]	5
CL	13.90 [5.60–23.06]	8	1.33 [1.18–3.77]	8	8.94 [1.36–19.19]	6
PTCE	13.20 [8.82–18.20]	5	1.43 [0.83–2.43]	5	9.86 [2.86–10.29]	4
ACP	9.77 [8.27–14.42]	4	1.44 [0.78–2.27]	4	4.51 [3.20–6.03]	3
PL	5.48[a] [2.69–7.01]	4	3.45 [2.10–7.59]	4	−1.34 [−3.30–3.51]	4

ACP = Ameroid constrictor placement. CL = Complete ligation; dB/s = Decibel per second; PL = Partial ligation; PTCE = Percutaneous transvenous coil embolization.

[a]Significantly lower than CL and PTCE.

group was significantly shorter than in the CL (P = 0.022) and PTCE groups (P = 0.049). The RR did not significantly differ between surgical treatments (P = 0.080). However, the RR in PL tended to be higher than the others. Although ΔHP-PV in PL tended to be shorter than the others, it did not differ significantly among the surgical treatments and control groups (P = 0.067).

Discussion

This study demonstrated that RT and RR in quantitative assessments of CEUS were significantly different between the low- and high-grade groups of the intrahepatic portal venous branches. In addition, RT was significantly different among the surgical procedures and among the shunt types (Pl vs Cl, and PL vs PTCE; LGP vs the others). On the other hand, ΔHP-PV in quantitative assessments of CEUS was significantly different between the shunt type (LGP vs the others). The RT, RR, and ΔHP-PV showed high sensitivity and specificity for diagnosing PSS in a previous report.[16] However, these parameters did not differ significantly between the control and PSS groups in this study. In a previous study,[16] the most common shunt types were splenocaval and splenoazygos shunts in 2 dogs each. Neurologic signs were most prevalent in dogs with splenocaval shunts[3] and dogs with splenocaval shunts may not develop intrahepatic portal veins preoperatively. Therefore, it was considered that CEUS in PSS was significantly different from that in normal dogs in that study. In contrast, in our study, 11/21 (52.4%) dogs had LGP, and 8/21 (38.1%) dogs had CL. The RT and ΔHP-PV in the LGP group were significantly longer than those in the other shunt types. In addition, the RT in the PL group was significantly shorter than that in the CL group. On the other hand, dogs with LGP and CL demonstrated the same values in these parameters compared with the control group. Therefore, our study showed different results, such that it may be difficult for CEUS to distinguish dogs with PSS from healthy dogs.

In dogs with LGP, respiration may affect the flow of blood in the shunt vessels. During inspiration, the portal blood volume flowing to the liver increases because of the diaphragm pressing on the shunt vessel and the increase in the pressure of CVC by intrathoracic pressure, which is thought to lead to the development of intrahepatic portal venous branches and the relative maintenance of intrahepatic portal blood volume in LGP. In addition, some reports[3,6] considered that SF in LGP was low and clinical signs were mild due to increased portal blood volume to the liver. Therefore, dogs with LPG have developed intrahepatic portal venous branches and increased intrahepatic portal blood volumes. Therefore, this study showed that all quantitative parameters in dogs with LGP were similar to those in the control group.

In this study, the RT and RR of PL were significantly different from those of CL. In addition, the ΔHP-PV on PL tended to be shorter than the others. This study demonstrated that the RT, RR, and ΔHP-PV in portal venous pressure during temporary occlusion and the ratio of increase in portal venous blood pressure by temporary occlusion of the shunt vessel were significantly correlated. Intraoperative measurement of portal venous blood pressure is one of the criteria for the degree of attenuation in animals undergoing CL or PL.[1] Therefore, CEUS showed the ability to estimate in PSS dogs needing PL before surgery. It has been reported that dogs that tolerate CL usually have better outcomes and are less likely to develop multiple acquired shunts than those that can only tolerate PL,[8,9,17,18] and it is clinically important to estimate those patients who require PL before surgery. Therefore, our study suggests that CEUS assessments using RT and RR could help in preoperative planning to predict the surgical procedure for canine extrahepatic PSS.

In the assessment of portovenography, the intrahepatic portal venous branch grade in preocclusion was greater in dogs that tolerated complete versus partial PSS attenuation.[13] In our study, all dogs with grade 1 in the CTA grading system underwent PL. In the intrahepatic portal venous branch grades, grade 1 RT differed significantly from grades 3 and 4. The RR in grade 1 showed a significant difference from grade 4. In addition, RT and RR in the low-grade group significantly differed from those in the high-grade group. Therefore, CEUS may reflect the grade of intrahepatic portal venous branches. Previous reports[15,16] have considered that increased hepatic arterial blood flow compensates for decreasing hepatic portal blood flow, leading to decreased RT and increased RR in dogs with PSS. Likewise, the dogs with grade 1 and the low-grade group showed decreased RT and increased RR than the other grades and the high-grade group, respectively. Therefore, RT and RR in dogs with PSS are suggested to reflect a decrease in hepatic portal blood flow.

This study has several limitations. First, we used a small sample size. Second, in our study, CEUS findings of the portal vein were not described in 4 dogs because of the gas in the stomach and duodenum. When the gas abundantly exists in the stomach and duodenum, CEUS might not be performed because the gas interferes with the ability to obtain clear ultrasound images of liver and portal vein. However, RT and RR in HP showed significant differences in surgical treatment and intrahepatic portal venous branch grades and were correlated with portal venous pressure. Therefore, these parameters are suggested to be of satisfactory clinical value. Third, the relationship between CEUS findings and hepatic histopathological findings was not evaluated in this study. Further studies may reveal the degree to which decreased intrahepatic portal venous branch development and hepatic arterial hyperplasia on histopathology could result in RT reduction and RR elevation in CEUS. Finally, the difference of CEUS findings between PSS and portal vein hypoplasia was not evaluated in this study.

In conclusion, this study showed that shunt type, surgical treatments, intrahepatic portal venous branch grades, and portal venous pressure affected the quantitative assessments of CEUS in canine PSS. Quantitative assessments of CEUS may be helpful in estimating intrahepatic portal vascularity in dogs with single extrahepatic PSS.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.