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Quantification of Doppler Indices, Contrast Ultrasound Enhancement Phases and Perfusion Parameters of Splenic Parenchyma in Healthy Dogs

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ABSTRACT

Background: The spleen can be affected by a variety of vascular problems. Affected tissue neovascularization in these conditions can be effectively analysed using imaging modalities such as Doppler and contrast enhanced ultrasonography (CEUS).

Methods: Nine healthy dogs were included in this clinical study comprising two breeds of Labrador Retrievers, German Shepherds, and Beagles and one breed each of Crossbred, Pug, and Pitbulls. Pulsed-wave Doppler indices and contrast-enhanced perfusion parameters of the splenic vasculature were obtained by performing spectral Doppler sonography and CEUS using a second-generation ultrasound contrast agent (Sonovue).

Result: The mean ± SE value of splenic vein's Doppler indices were 10.94±2.04 cm/s (PSV), 6.92±1.28 cm/s (EDV), 6.44±1.57 cm/s (MV), 0.35±0.05 (RI) and 0.75±0.17 (PI), while the indices of splenic artery were 31.21±3.12 cm/s (PSV), 8.95±1.63 cm/s (EDV), 13.98±1.95 cm/s (MV), 0.71±0.04 (RI), 1.75± 0.24 (PI). Arterial and venous phases were visible on a CEUS of the splenic parenchyma. The mean ± SE value of the contrast enhanced splenic perfusion parameters (measured in seconds) were as follows: arrival time = 7.00 ± 0.33; time to initial peak = 15.05 ± 0.46; time to final peak = 28.92±1.06; decline time = 97.89±2.82; washout time = 152.22± 9.10.

Key words: Contrast enhanced ultrasonography, Doppler velocity, Splenic perfusion parameters, Zebra view.

INTRODUCTION

The spleen is a secondary lymphoid organ supplied with blood in mammals. Because the spleen performs immunological, circulatory, lymphatic, and hematopoietic processes, it is crucial to precisely diagnose splenic diseases (Pîrvu *et al.,* 2021). The primary clinical diagnostic imaging modality of choice in splenic diseases is ultrasound because of its non-invasiveness, real-time imaging capabilities and lack of hazardous radiation. Apart from diagnosing the splenic condition, it also helps in the formulation of an accurate and effective treatment strategy (Farooq *et al.,* 2018).

In recent times, conventional ultrasonography has greatly improved its diagnostic capabilities with the use of doppler and specific ultrasonic contrast agents (UCA). Doppler ultrasonography is helpful for splenic mass characterization (Sharpley *et al.,* 2012), assessment of accessory spleens following splenectomy (Herneth *et al.,* 2001), diagnosis of splenic vein thrombosis and in assessing tissue neovascularization in splenic diseases, neoplasia and torsion, the latter of which is defined by the lack of blood flow (Gil *et al.,* 2015). Doppler and B-mode ultrasonography can provide real-time information on the vascular architecture and hemodynamic properties of blood vessels (Carvalho *et al.,* 2008), but are unable to detect blood flows in smaller vessels (Singh *et al.,* 2023). Because microbubble contrast enhanced ultrasonography fills in microvascular spaces, it can identify blood even in smaller vessels (Rafailidis *et al.,* 2020).

Ultrasonic microbubble contrast agents differ from other radiography contrast agents in that they are able to remain entirely within the intravascular space after injection. These days, CEUS is widely used to detect and characterize liver lesions. Splenic ultrasound contrast imaging is a promising field, as it can also hold and collect ultrasonic contrast microbubbles, much like the liver (Omar and Freeman, 2016). Contrary to computed tomography, CEUS does not expose the patient to radiation and can be carried

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out on an un-sedated animal (Sammon *et al.,* 2012; Lim *et al.* 2004). Furthermore, CEUS can identify lesions whose densities are similar to the organ's average ultrasonic density (Piscaglia and Bolondi, 2006).

Splenic abnormalities that are not visible with B-mode ultrasonography can be successfully detected using the Doppler and CEUS. Every vessel has its own unique Doppler waveform and perfusion characteristics. To diagnose splenic anomalies early and accurately, sonologists and clinicians must have a thorough understanding of normal and aberrant waveforms, doppler indices and perfusion parameters. The goal of the study is to address the existing shortcomings in veterinary practice with the use of Doppler and CEUS on the splenic vasculature. The literature is devoid of data regarding the Doppler blood flow indices and CEUS perfusion parameters of the spleen in clinically healthy dogs. An overview of the Doppler and CEUS was provided by prior studies. Since these blood flow characteristics might be used as canine reference values, the current study focused on providing detailed information on their quantification. The Reference values can be used in clinical setting in early diagnosis of splenic abnormalities.

MATERIALS AND METHODS

Nine healthy dogs, five males and four females, weighing 21.67±3.14 kg (7.5 to 35 kg) and aged 4.81±1.02 years (9 months to 10 years) were brought to the Multi-Speciality Veterinary Hospital, GADVASU, Ludhiana, India, between 2021 and 2023 for the purpose of this study. Once the owners gave their consent, the study used randomly selected healthy dog breeds (two each of Labrador Retriever, German Shepherd, and Beagle and one each of Crossbred, Pug, and Pitbull) brought in for routine veterinary health check-ups. For the use of contrast agents in these dogs, IAEC approval was obtained under number GADVASU/2021/IAEC/60/12. The lack of haematological and biochemical abnormalities, the absence of a parenchymal lesion on B-mode ultrasonography, and the absence of any concurrent disease at the time of assessment served as the inclusion criteria for healthy dogs.

All the dogs were restrained on the ultrasound table in dorsal recumbency without any sedation. The splenic vessels were identified utilizing B-mode and colour Doppler exams using the Philips Affiniti 70G ultrasound machine. The splenic hilus was taken as the reference point during the scanning procedure. The splenic artery and vein enter the splenic parenchyma at the hilus and then divide into various branches. The Doppler indices of these vessels were then shown using spectral Doppler ultrasonography. A doppler angle of no more than 60 degrees and a sample volume gate with a width of 1.5 to 2.0 mm were employed. A reduced sample volume gate (0.5-1.0 mm) was utilized in the evaluation of splenic artery because of its smaller diameter. The ultrasound machine recorded the clear waveform of the splenic vessels, including the splenic artery and vein and computed their corresponding spectral Doppler indices, including velocities such as peak systolic velocity (PSV), end-diastolic velocity (EDV), resistivity index (RI) and pulsatility index (PI). Mean velocity (MV), also referred to as average velocity (MV), was calculated using the following formula:

$$MV = \frac{PSV - EDV}{PI}$$

The calculation was made using the mean of three measurements of these indices, preferably from different vessel branches of splenic artery and splenic vein.

All the dogs were given a bolus injection of Sonovue (Bracco Suisse SA, Geneva, Switzerland). A second-generation ultrasound contrast agent consisting of gas-filled micro bubbles containing sulfur hexafluoride gas encapsulated in a phospholipid shell, at a rate of 0.04 ml/kg body weight, into the cephalic vein. A quick bolus of 5 ml of saline flush was administered after that. A C1-5 low frequency transducer was used to scan the splenic parenchyma utilizing splenic hilus as the reference point.

The entire scanning process took three to five minutes to record and all of the images and videos were periodically collected into DICOM files. After comparing the images obtained before and after the application of contrast agent, the interpretation was finished. Next, the contrast-specific software was used to examine the DICOM video in order to quantify the contrast-enhanced perfusion parameters. Motion correction option was chosen in order to reduce the impact of movement while analysing the DICOM video, and a region of interest was placed in the splenic parenchyma (Fig 1). The contrast software produced a graph that was used to determine the contrast perfusion parameters, including arrival time, or the point at which the contrast agent appears, time to peak (initial), or the instant the intensity begins to rise after arrival time, time to peak (after), or the instant the intensity peaks after the initial rise, decline time, or the instant the intensity starts going down and wash out time, or the instant the contrast agent begins to wash out of the organs. One extra region of interest for the splenic artery was chosen in order to measure the phases of splenic contrast ultrasonography. Using Microsoft Excel, the quantitative data was displayed as Mean±SE.

RESULTS AND DISCUSSION

In the pre sent study, the rate of blood flow to the spleen and its related indices viz PSV, EDV, MV, PI and RI were evaluated in healthy dogs as shown in Table 1. These parameters are frequently used in humans to detect hepatic and renal pathologies (Gonul *et al.,* 2020). In the pre sent case, the splenic artery showed a low resistance, parabolic laminar blood flow pattern characterized by a pulsatile forward flowing waveform above the baseline having typical systolic-diastolic components with spectral widening and

lack of a spectral window. Similar findings were reported by Szatmari *et al*. (2001). On the other hand, splenic vein had a consistent laminar blood flow pattern below the baseline with a flat, non-pulsatile forward flow as reported earlier by Finn-Bodner and Hudson (1998). Phasicity was occasionally noticed, which was explained by panting and elevated abdominal pressure in dogs. The mean±SE value of diameter of splenic vein at hilus was 0.49±0.04 cm.

The mean±SE values of Doppler indices (PSV, EDV, MV, RI and PI) for splenic artery and splenic vein of dogs were represented in Table 1. Most recently, increase in splenic impedance indices (RI and PI) has also been observed in the setting of portal hypertension (Cançado *et al.* 2007). The mean±SE value of Doppler indices of splenic artery were RI (0.71±0.04), PI (1.75±0.24), PSV (31.21±3.12 cm/s), EDV (8.95±1.63 cm/s) and MV (13.98 ± 1.95 cm/s). The overall mean ± SE value of Doppler indices of splenic vein were RI (0.35±0.05), PI (0.75±0.17), PSV (10.94±2.04 cm/s), EDV (6.92±1.28 cm/s) and MV (6.44± 1.57 cm/s). Similar values for doppler indices of splenic artery were reported in dogs (RI=0.71±0.14; Maronezi *et al.* 2015), rabbits (RI=0.7±0.03, PI= 1.02±0.35, PSV= 25.41±2.56 cm/s; Maher *et al.,* 2020) and donkeys (RI= 0.74±0.005, PI= 1.31±0.03, MV= 9.46±0.22 cm/s: Fouad *et al.,* 2018).

Splenic artery RI has also been used to diagnose renal artery stenosis (Grupp *et al.,* 2018), whereas splenic PI has been established as one of the most dependable indicators in Doppler ultrasonography for detecting splenic congestion in patients with right-sided or congestive heart failure (Bolognesi *et al.,* 2012) and for assessing hepatic fibrosis in patients with chronic hepatitis (Liu *et al.,* 2023). Furthermore, vascular indices such as PSV and EDV could be useful in the therapeutic monitoring of sub clinical haemoparasitic diseases such as subclinical Ehrlichiosis in dogs, wherein, lowered splenic artery EDV values (5.25 ±4.66 cm/s) and PSV values (22.59±8.07 cm/s) were observed (Maronezi *et al.,* 2015).

Enhancement of the spleen during the CEUS examination was very heterogeneous at the beginning of imaging. Spleen on CEUS examination showed three phases namely the arterial phase, venous phase and the late venous phase (Fig 2). The arterial phase was marked by the influx of contrast agent into the splenic parenchyma followed by mild enhancement of splenic parenchymal arteries initially (Fig 2a).The splenic arteries enhanced rapidly and took on a radiating appearance from the hilus (Fig 2b). This radiating appearance of splenic arteries from the hilus at the end of the arterial phase was described as the “zebra” view by Haers *et al*., (2009), Canejo–Teixeira *et al.* (2022) and Maronezi *et al*., (2015). This was followed by mild enhancement of the area around the enhanced splenic arteries (arterial peak phase) (Fig 2c). During this phase, there is non-homogenous enhancement of the parenchyma. The arterial phase was followed by the venous phase wherein the entire splenic parenchyma was markedly enhanced (Fig 2d).

The third phase (late phase) was marked by decline of splenic parenchymal enhancement (Fig 2e) and persisted until the entire contrast agent was washed-out from the splenic parenchyma and splenic parenchyma becomes non-enhanced (Fig 2f). The mean±SE of the contrast perfusion parameters (in seconds) of spleen is shown in Table 2.

The overall wash in time of contrast agent in canine spleen was 7±0.33 seconds. At 15.05±0.46 seconds, there was enhancement of the parenchyma surrounding these vessels (initial peak), followed by a more progressive enhancement of remainder of the spleen at 28.92±1.06 seconds (venous) that lasted up to 97.89±2.82 seconds. Similar wash-in time (10±2 seconds) was reported by Morabito *et al*., (2021). Ohlerth and O’Brien (2007) also

Table 1: Mean ± SE value of Spectral Doppler indices of splenic vessels in healthy dogs.

Vessel	PSV (cm/s)	EDV (cm/s)	MV (cm/s)	RI	PI
Splenic artery	31.21±3.12	8.95±1.63	13.98±1.95	0.71±0.04	1.75±0.24
Splenic vein	10.94±2.04	6.92±1.28	6.44±1.57	0.35±0.05	0.75±0.17

PSV- Peak systolic velocity; EDV- End diastolic velocity; MV- Mean velocity; RI- Resistivity index; PI- Pulsatility index.

Table 2: Mean ± SE value of contrast perfusion parameters (in seconds) in different phases of splenic contrast ultrasonography.

Arterial phase		Venous phase	Late phase	
Arrival phase or SAEi	Initial parenchymal Peak enhancement or SAE_p phase	peak parenchymal enhancement phase or SVE_p phase	Decline phase	Washout phase
7.00±0.33 sec	15.05±0.46 sec	28.92 ±1.06 sec	97.89±2.82 sec	152.22±9.10 sec

SAE_i - Splenic artery initial enhancement; SAE_p - Splenic artery peak enhancement; SVE_p - Spleni vein peak enhancement; Sec- Seconds.

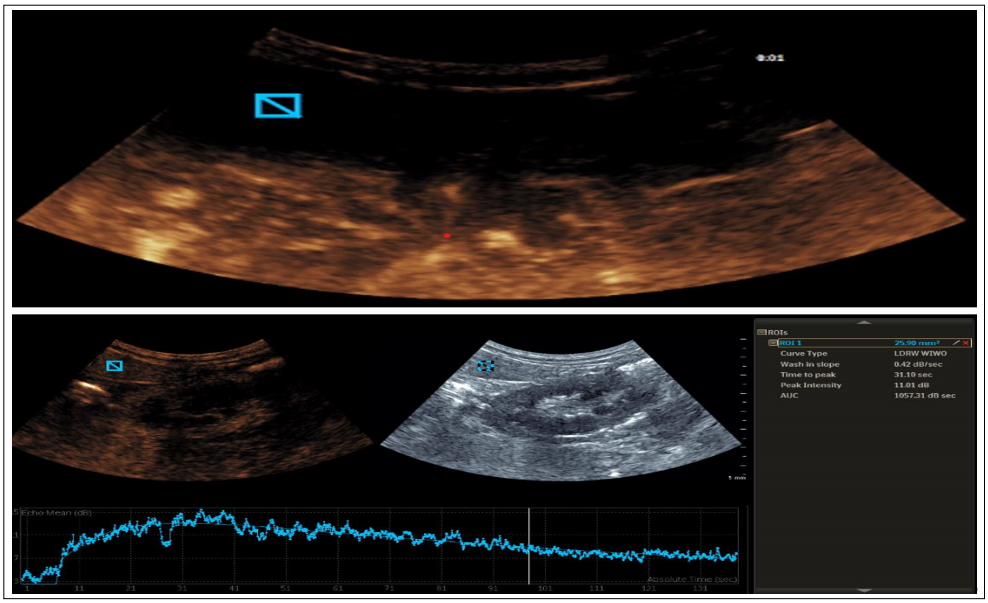
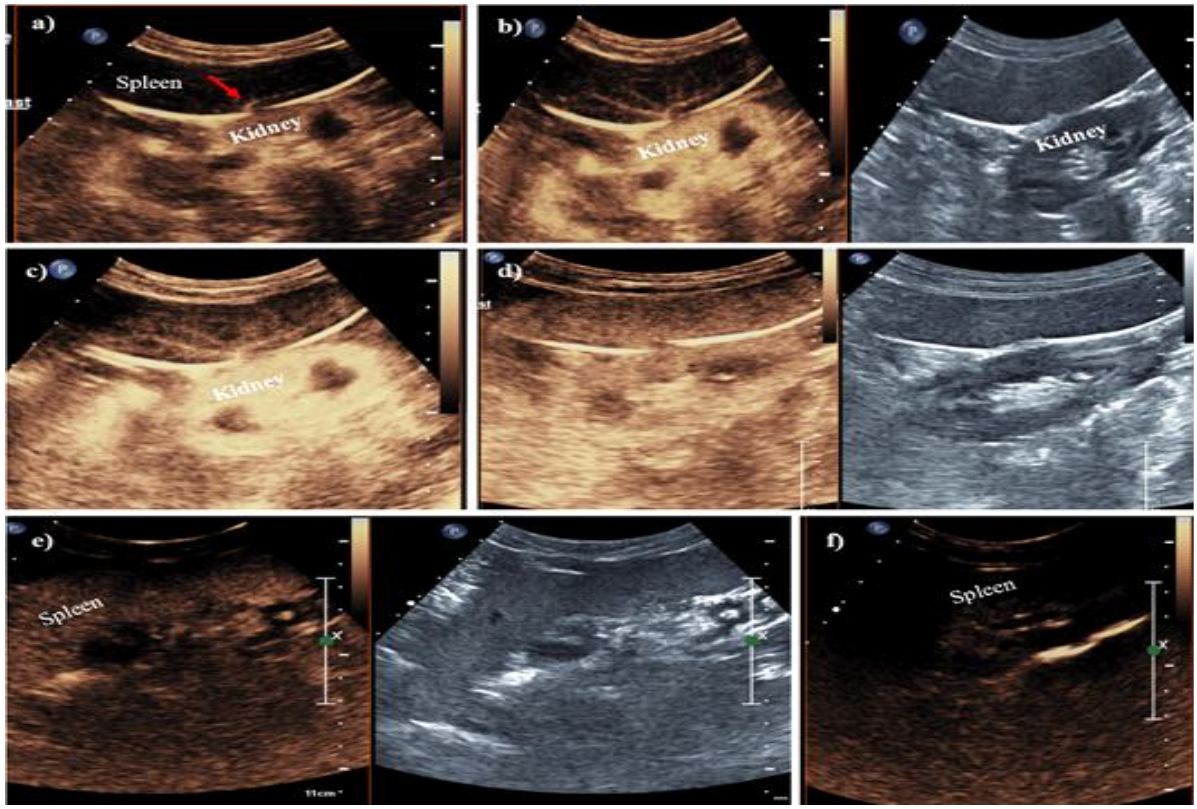


Fig 1: Placement of region of interest in the splenic parenchyma for quantification of contrast-enhanced perfusion indices.



a) Arrival phase or arrival time, b) Zebra pattern of splenic arteries, c) Initial parenchymal peak enhancement phase or peak arterial phase, d) Final parenchymal peak enhancement phase or peak venous enhancement phase characterized by homogenous enhancement of spleen, e) Decline phase characterized by declining of the parenchymal enhancement and f) Wash-out phase characterized by complete loss of the parenchymal enhancement.

Fig 2: CEUS of canine spleen through the transabdominal approach in the left cranial quadrant.

reported similar time to initial peak and final peak (13.3 seconds and 25.6 seconds) during CEUS examination of normal canine spleen.

Splenic pathologies are associated with significant blood flow changes. Doppler and CEUS have the ability to identify these changes in the initial stages of affection, even when the B-mode sonography is unable to detect any abnormality. CEUS has been used earlier to diagnose a variety of clinical affections, including accessory spleen, splenic haemangiomas, vascular splenic pathologies such as splenic infarction, splenic abscess (Görg ,2007), Canine ehrlichiosis (Maronezi *et al.,* 2015) and differentiation of benign and malignant focal splenic lesions (Rossi *et al.,* 2008). Hypo-echogenicity of the lesion as compared to the parenchymal echogenicity in the wash-out phase in conjunction with the presence of tortuous feeding arteries was highly suggestive of malignancy.

CONCLUSION

The spleen serves as the major location for a variety of affections like neoplasia, splenic torsion, splenic infarction, etc. These affections change the normal splenic Doppler indices and perfusion parameters. To differentiate a normal spleen from an abnormal one, it would be helpful to establish and standardize typical values for doppler indices and contrast perfusion parameters in healthy dogs. The present study does a preliminary work and forms a base for further standardization which requires the involvement of a large sample size.

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Conflict of interest

The authors don't have any conflict of interest.

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