EFFECT OF SEDATION ON CONTRAST-ENHANCED ULTRASONOGRAPHY OF THE SPLEEN IN HEALTHY DOGS

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Contrast-enhanced ultrasound of the spleen enables the dynamic assessment of the perfusion of this organ, however, both subjective and quantitative evaluation can be strongly influenced by sedative agent administration. The purpose of this prospective, experimental study was to test effects of two sedative agents on splenic perfusion during contrast-enhanced ultrasound of the spleen in a sample of healthy dogs. Contrast-enhanced ultrasound of the spleen was repeated in six healthy Beagles following a cross-over study design comparing three protocols: awake, butorphanol 0.2 mg/Kg intramuscular (IM), and dexmedetomidine 500 $\mu g/m^2$ IM. After intravenous injection of a phospholipid stabilized sulfur hexafluoride microbubble solution (SonoVue®, Bracco Imaging, Milano, Italy), the enhancement intensity and perfusion pattern of the splenic parenchyma were assessed and perfusion parameters were calculated. Normal spleen was slightly heterogeneous in the early phase, but the parenchyma was homogeneous at a later phase. Sedation with butorphanol did not modify perfusion of the spleen. Dexmedetomidine significantly reduced splenic enhancement, providing diffuse parenchymal hypoechogenicity during the entire examination. Measured parameters were significantly modified, with increased arrival time (AT; (< 0.0001) and time to peak (TTP; $P < 0.0001$), and decreased peak intensity (PI; $P = 0.0108$), wash-in ($P = 0.0014$), and area under the curve (AUC; $P = 0.0421$). Findings supported the use of butorphanol and contraindicated the use of dexmedetomidine as sedatives for splenic contrast ultrasound procedures in dogs. Short-term and diffuse heterogeneity of the spleen in the early venous phase was determined to be a normal finding. © 2016 *American College of Veterinary Radiology.*

Key words: contrast-enhanced ultrasound, dog, perfusion, sedation, spleen.

Introduction

IN THE LAST DECADE, there was an increased use of contrast-enhanced ultrasound in veterinary medicine.[1,2] The possibility to study organs perfusion in real time opens interesting potential clinical applications, already explored in human medicine. These include not only a better detection and characterization of focal parenchymal lesions, but also the evaluation of diffuse organs changes, intravascular diseases, and pre- and posttreatment perfusion during ablation procedures.[3–5]

The spleen is a complex organ with peculiar vascular architecture and high incidence of pathology in the dog.[6] Contrast-enhanced ultrasound has been recently investigated as a potential imaging method to improve diagnosis and characterization of splenic diseases. The normal splenic perfusion pattern and dynamics in dogs has been described.[7,8] Small splenic arteries become visible during first seconds. During the early venous phase, the parenchyma progressively enhances, sometimes heterogeneously, until the peak is reached. In the wash-out phase, a progressive and homogeneous decrease of the opacification follows. First, studies have been performed to improve characterization of focal splenic lesions, however, their results are for some aspects controversial,[7,9–12] therefore more research in the near future is expected. When developing a protocol for a dynamic imaging study of the vascular compartment, an important issue is the state of the cardiovascular system, which can be strongly influenced by sedative drugs administration. Sedation if often needed in uncooperative patients, therefore, it is important to know which protocols can be used without altering perfusion or having significant effect on the evaluated dynamic parameters.

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Splenic congestion is frequently present during sedation or anesthesia,[13] and this could potentially influence the flow pattern of contrast medium, with altered subjective interpretation and quantitative results.[14] Perfusion changes of abdominal organs have been reported in a high percentage of anesthetized cats compared to awake animals[15] and in dogs after administration of dexmedetomidine.[16] In a recent study, effect of anesthesia with propofol and sedation with butorphanol on contrast-enhanced ultrasonography of normal feline kidney was evaluated.[17]

To our knowledge, comparison between different sedative agents on splenic perfusion during contrast-enhanced ultrasound in dogs has not been investigated. Therefore, the purpose of this study was to investigate and compare the effect of butorphanol and dexmedetomidine on the normal canine splenic perfusion.

Material and Methods

The study was designed as a prospective crossover experimental study. Six adult healthy Beagles were included, two females and four males of 6 years old and a mean weight of 11.51 ± 1.58 kg. This study was approved by the Local Ethical Committee of the Ghent University (EC2012/201). Dogs were housed by groups of two in cages of 2.14 × 1.84 m, divided in two parts. They had baskets and boards to rest on. Temperature (15–21°C) and ventilation and humidity were controlled daily. They received dry food, and clean drinking water ad lib. They had access to an outdoor field several hours a day in stable groups of eight dogs, and were walked daily with students or personnel volunteers of the veterinary faculty of Gent.

The dogs were declared healthy based on a normal clinical examination and abdominal ultrasonography performed by a second-year imaging resident (CF), and normal blood count and biochemistry profile. Each dog was scanned three times by the same operator (CF), a second-year imaging resident, leaving a wash-out period of 1 week between the scans. The three different protocols were randomly assigned as (1) no sedation group (NSG), (2) butorphanol group (BG; Dolorex®, Intervet Schering-Plough, USA, 0.2 mg/kg IM), or (3) dexmedetomidine group (DG; Dexdomitor®, Pfizer Animal Health, USA, 500 μg/m^2 IM). The same experimental setup was repeated 2 weeks later, resulting in six sonographic sessions performed in each dog. All dogs were fasted for 12 h before the experiment. The hair of the ventral abdomen was clipped and coupling gel was applied. Contrast-enhanced ultrasound was performed 20 min after administration of the sedative agent.

The left cranial abdomen was scanned with an ultrasound equipment with specific contrast software (Philips iU22 system, Bothell, WA) and a 5–12 MHz linear probe. A longitudinal view of the spleen, including the hilar vessels was obtained with similar orientation in all animals. System settings were optimized for the contrast study with a mechanical index of 0.09 and single focal zone fixed in the distal field. Power and time-gain compensation were adjusted so that the fundamental signal of the parenchyma was completely suppressed and only the thin interface of the spleen capsule was partially visible. The position of the probe and the setting parameters were maintained unchanged for the entire examination and were very similar for the subsequent/following sessions. A bolus of intravenous phospholipid stabilized sulfur hexafluoride microbubble solution (SonoVue®, Bracco Imaging, Milano, Italy) was injected through a cephalic vein catheter at the dose of 0.03 ml/kg, followed by a 5 ml saline flush using a three-way stopcock. The timer was activated at the moment of starting the injection and the splenic perfusion was observed in real time. The probe was kept exactly in the same position for at least 2 min. In total, each dog received three injections of contrast medium at 5 min interval. The first injection was not used for interpretation, but to optimize the system settings. In between the injections, the residual microbubbles were destroyed by increasing acoustic power and scanning the left cranial abdomen. Absence of visualization of signal from microbubbles in the abdominal aorta indicated the destruction of almost all circulating microbubbles. Videos were recorded digitally after each injection in DICOM format.

Subjective qualitative analysis was performed and the following parameters were evaluated: time to homogeneity (TTHo), enhancement intensity (weak, medium, intense), and distribution pattern (homogeneous vs. heterogeneous) of the contrast medium during the phase of increasing echogenicity (wash-in), maximum enhancement (peak), and decreasing echogenicity (wash-out). Presence or absence of hilar-enhancing vessels was also recorded. TTHo was defined as the intervals (seconds) between the injection and the time when the spleen was judged homogeneous. Enhancement intensity was defined weak if the splenic parenchyma had a minimal increase in echogenicity during the examination and remained clearly hypoechoic compared to the enhancing structures surrounding the spleen like the perihilar vessels and the adjacent tissue. Enhancement was considered intense when the spleen was clearly hyperechoic after contrast administration, with a change in echogenicity similar or more intense compared to the adjacent structures. Medium enhancement was defined as an intermediate situation compared to the two previously described. The distribution pattern was considered homogeneous if all parts of the parenchyma enhanced contemporarily and with similar echogenicity, and heterogeneous if the spleen showed a mottled appearance with adjacent enhanced and nonenhanced areas.

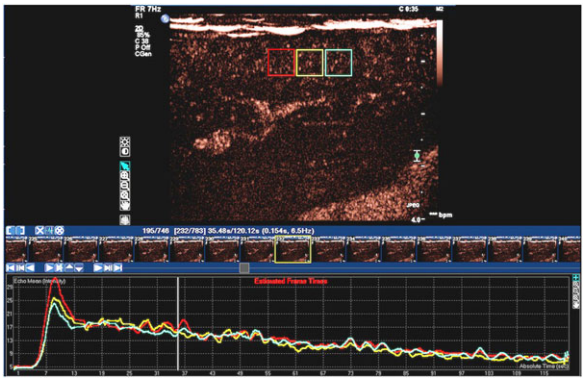

FIG. 1. Placement of the three regions of interest (ROIs) within the splenic parenchyma for quantitative analysis of splenic enhancement using the QLAB commercial software program in a dog. The three ROIs have the same size and shape and are located at the same depth within the splenic parenchyma. A time–intensity curve is generated from each ROI.

A commercial software program (QLAB, Philips, AG) was used for quantitative computerized analysis. A single observer (CF) drew three squared regions of interest (ROIs) of similar area and located at the same depth on the ultrasound image within the splenic parenchyma (Fig. 1). A numeric value representing mean pixel intensity for each of these ROI was determined over 120 s (at seven frames per second). Time-intensity curves were generated and a given curve-fit (the γ variate) was applied to smooth the curve. Following perfusion parameters were calculated: mean baseline intensity (MBI), peak intensity (PI), arrival time (AT), time to peak (TTP), wash-in (upslope), wash-out (downslope), and area under the curve (AUC). To calculate average up- and downslope, the part of the curve with values above 10% of the baseline and up to 85% of the peak enhancement was used.

Statistical tests were selected and performed by a statistician (LD). Statistical analysis was based on a linear mixed model with sequence and sedation protocol (NSG, BG, or DG) as categorical fixed effects and dog as random effect. *F* tests were performed to assess the effect of sequence and sedation protocol on the values of the different perfusion parameters previously mentioned (MBI, PI, AT, TTP, wash-in, wash-out, AUC). All tests were based on a global significance level of 5%. The significance level for multiple comparisons between the three sedation protocol groups was adjusted using Bonferroni's technique.

Results

Sedation level was considered satisfying for both protocols, however, it was more superficial in BG compared to DG and manual restraint was often necessary in BG. The perfusion pattern observed in NFG and BG was similar (Fig. 2). During the first seconds, rapid and intense enhancement of thin splenic arteries arising from the hilus

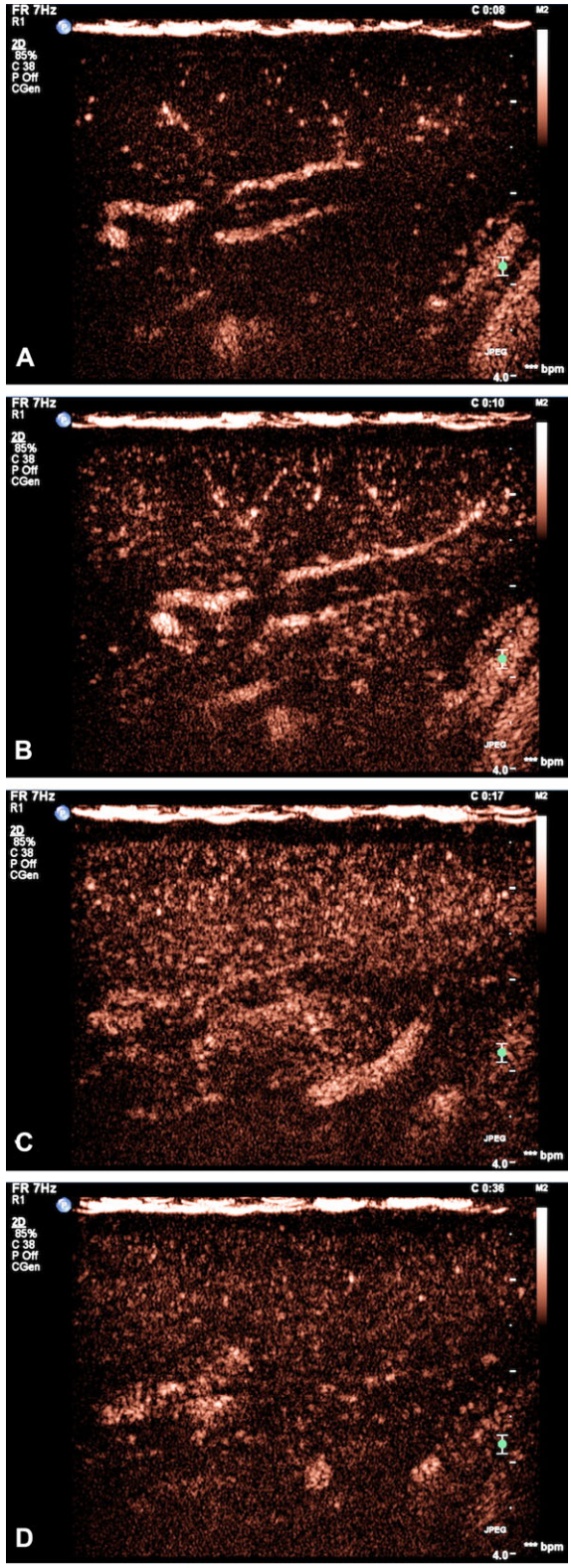

FIG. 2. Contrast-enhanced examination in a dog sedated with butorphanol. Splenic arteries arising from the hilus show early and intense enhancement 8 s after injection (A). Subsequently, the splenic parenchyma has heterogeneous enhancement (B) gradually becoming homogeneous 17 s after injection (C). Gradual and slow decrease of enhancement is observed during the wash-out phase (D).

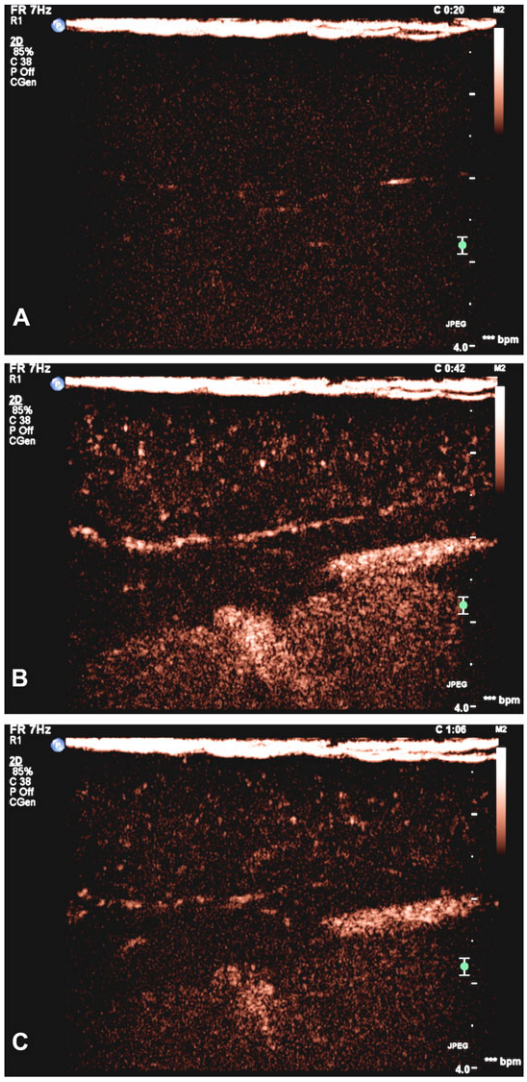

FIG. 3. Contrast-enhanced examination of the same dog sedated with dexmedetomidine. Twenty seconds after injection, the spleen shows no enhancement (A). The peak enhancement occurs 42 s after injection (B). Gradual decrease of enhancement occurs during the wash-out phase (C). The enhancement is subjectively weak during the entire procedure in comparison with Fig. 2.

was observed, followed by heterogeneous enhancement of the parenchyma in the early venous phase. Splenic tissue became gradually uniformly homogeneous, with a mean TTHo of 17.41 s ± 6.53. The splenic parenchyma change was uniform, independently from the relationship with the vessels. At peak, the parenchyma was hyperechoic and the enhancement was intense. During the wash-out phase, a gradual, homogeneous slow decrease of enhancement was observed. In DG (Fig. 3), the contrast medium distribution was slower. The whole organ was diffusely hypoechoic during the first 30 s in all dogs and enhancement was estimated to be weak and homogeneous during the entire procedure. The differences in time–intensity curves between the three groups are illustrated in Fig. 4.

TABLE 1. Contrast-Enhanced Ultrasound Perfusion Parameters Measured in the Spleen of Nonsedated Healthy Beagle Dogs and in Dogs with Butorphanol and Dexmedetomidine ($n = 6$)

	NSG	BG	DG
MBI	3.62 ± 0.19	3.66 ± 0.19	3.58 ± 0.19
PI	17.86 ± 1.92	16.48 ± 1.92	9.19 ± 1.92§
AT	8.77 ± 1.16	7.62 ± 1.16	30.08 ± 1.16*
TTP	12.03 ± 1.51	11.48 ± 1.51	39.41 ± 1.51*
Wash-in	4.54 ± 0.59	4.25 ± 0.59	1.15 ± 0.59§
Wash-out	−0.09 ± 0.01	−0.11 ± 0.01	−0.06 ± 0.01
AUC	803 ± 108	721 ± 108	408 ± 108§

MBI, mean baseline intensity; PI, peak intensity; AT, arrival time; TTP, time to peak, wash-in (upslope), wash-out (downslope); AUC, area under the curve; NSG, no sedation group; BG, butorphanol group; DG, dexmedetomidine group. In DG, AT and TTP were significantly different from NSG and BG with a P-value < 0.001 (*); similarly, PI, wash-in and AUC were reduced (P-value < 0.05 [§]).

Table 1 summarizes the calculated perfusion parameters values. MBI was similar in the three groups of dogs, confirming that the machine setting was equivalent. There were no significant differences for any of the perfusion parameter values between NSG and BG ($P = 0.7629$, 0.964, 0.8695, 0.936, 0.4009, and 0.8547 for the parameters AT, TTP, PI, wash-in, wash-out, and AUC, respectively). The AT and TTP mean values increased significantly after sedation with dexmedetomidine, with mean values of 30.08 ± 1.16 s for AT ($P < 0.0001$) and 39.41 ± 1.51 s ($P < 0.0001$) for TTP. The PI, wash-in, and AUC mean values significantly decreased ($P = 0.0108$, 0.0014, and 0.0421; Table 1).

Discussion

Knowledge of the effects of commonly used sedative agents on contrast procedure is important to avoid misinterpretation of iatrogenically induced abnormal perfusion patterns. This study demonstrates that butorphanol does not cause either subjectively observable or objectively measurable effects during contrast-enhanced ultrasound of the normal canine spleen. The agonistic interaction with the central nervous opiate receptor site, predominant at k-receptor and only partial at μ-receptor, results in systemic analgesic effects without significant changes of myocardial contractility and vascular tone. No significant hypotension, visceral congestion, and organomegaly are expected.[18]

Dexmedetomidine is a specific and selective alpha-2 adrenoreceptor agonist. By binding to the central presynaptic alpha-2 adrenoceptors, it inhibits the release of norepinephrine, terminate the propagation of pain signals and causes sedation. Cardiovascular effects of dexmedetomidine are typically associated to a biphasic blood

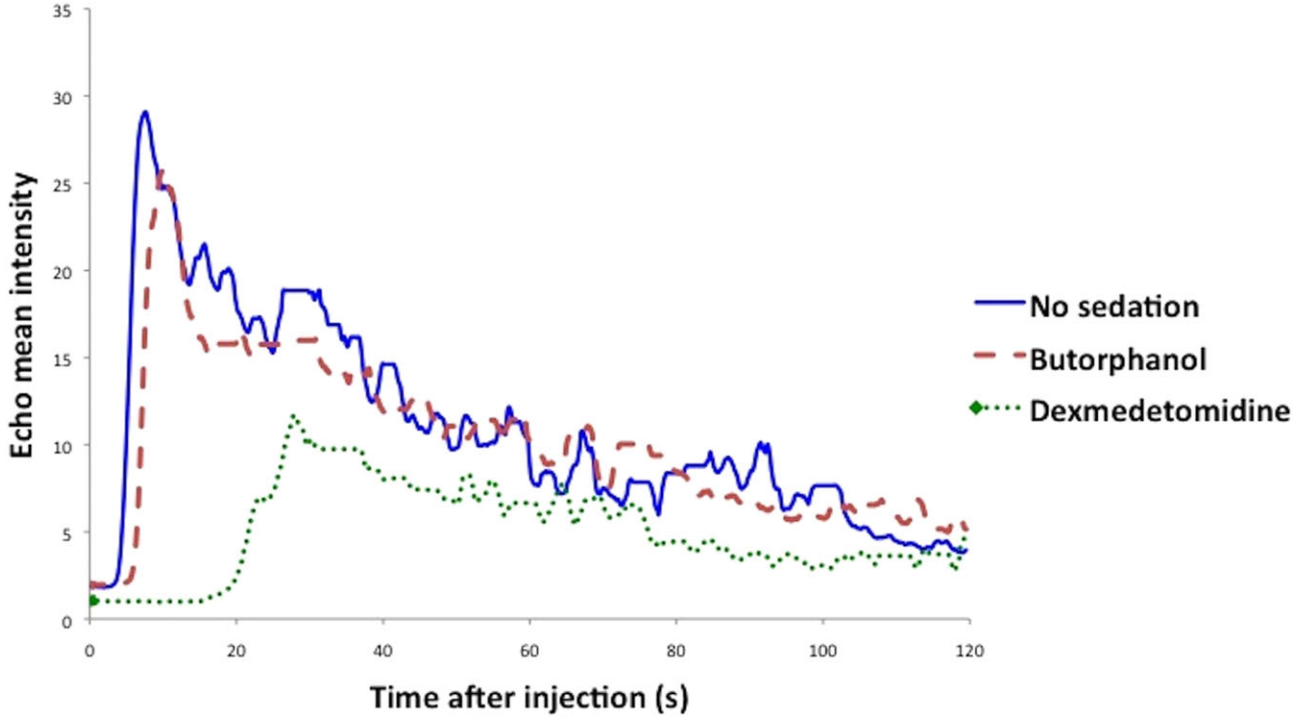

FIG. 4. Time–intensity curves of the three groups of dogs. No sedation (NSG) and butorphanol (BG) groups were overlapping, with a rapid and intense peak reached few seconds after the injection. In dexmedetomidine dogs (DG), splenic enhancement was slow and weak, AT and TTP showed a threefold increase compared to NSG and BG.

pressure response.[19,20] Initially, activation of peripheral postsynaptic alpha-2 adrenoreceptors leads to peripheral vasoconstriction and transitory hypertension. After that, a further decreased arterial blood pressure and activation of presynaptic alpha-2 adrenoreceptors block sympathetic activity, with consequent decreased cardiac output and reduced perfusion in peripheral organs.[19] In a recent study, the effect of dexmedetomidine on the contrast-enhanced ultrasound flow parameters measured in the kidney, spleen, small intestine, and liver was evaluated in dogs.[16] Concerning the spleen, dexmedetomidine administration resulted in longer AT and TTP compared to awake animals, whereas PI was similar.[16] In our DG dogs, the spleen clearly showed a very low enhancement, as judged both by subjective evaluation and objective analysis, according with secondary hypoperfusion and reduced peripheral blood flow. Reasons for the different results can only be speculated, based on the different design of the two studies, and could be related to the different injected contrast dose and timing of contrast administration. In a clinical setting, the hemodynamic consequences must be considered when dexmedetomidine is used and splenic hypoperfusion changes must be interpreted with caution. On the other hand, true hypoperfused lesions could be easily missed with a false-negative result.

In NFG and BG, heterogeneity of the splenic parenchyma in the early venous phase was observed. This pattern was previously mentioned in dogs after injection of SonoVue.[7,8] In a recent study in cats,[21] a heterogeneous pattern has been also described in the feline spleen, especially marked and long in anesthetized animals. However, the timing and characteristic of this heterogeneous pattern seems to be different between dogs and cats. First, the heterogeneous appearance of the canine spleen disappears faster compared to cats. In dogs, after a mean of 17.41 s, the spleen is homogeneous, therefore, TTHo is close to half compared to the 25–30 s reported for awake and anesthetized cats.[21] Second, the canine heterogeneity pattern is uniform, without different areas of enhancement as reported in the feline spleen.[21] Third, the appearance and timing of heterogeneity during contrast distribution is similar in awake animals and in dog sedated with butorphanol and is not observed in dogs after dexmedetomidine administration. Reasons for the species difference remain unclear because of the different type of sedation and study design. However, it is interesting to remember that studies on microcirculatory pathways of the spleen demonstrated that feline and canine spleen differs in vascular architecture and flow control mechanisms.[22,23] Dogs have a sinusal type of spleen with an extensive system of interconnected venous sinuses, directly communicating with arterial capillaries. In the nonsinusal feline spleen, blood circulation appears to be entirely open, with arterial capillaries ending in the reticular space of the red pulp, and no direct arteriovenous

connections are present. These findings are potentially correlated with the different behavior during contrast ultrasound. Independently from the cause, short and uniform heterogeneity in the early venous phase is normal in dogs and this must be considered when abnormal perfusion patterns are suspected.

The main limitation of this study is that only two sedative agents were compared. Authors chose these two agents because, in the clinical setting, these are the most commonly used protocols when a superficial and quick sedation is needed for an ultrasound contrast study. More research is needed to test the effects of other drug combinations on contrast ultrasound characteristics of the normal canine spleen.

In conclusion, findings indicated that sedation with butorphanol did not influence the qualitative and quantitative splenic perfusion pattern during contrast-enhanced ultrasound for a sample of healthy Beagle dogs. Dexmedetomidine significantly reduced splenic enhancement and measured perfusion parameters. Authors recommend that dexmedetomidine be avoided for canine patients scheduled to have contrast-enhanced ultrasound of the spleen.

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