Review of clinical characteristics and applications of contrast-enhanced ultrasonography in dogs

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Ultrasonography is a noninvasive imaging tool that can be used to assess the size, shape, parenchymal texture, and vascularity of various organs. However, a US image provides low contrast because of the similar acoustic impedances of soft tissues. The accuracy of diagnostic US can be improved by IV injection of gas microbubbles as vascular contrast agents. Ultrasonographic contrast agents consist of tiny, gas-filled microspheres stabilized by an outer shell. These microbubbles (1 to 7 μm in diameter) are smaller than RBCs, eliminating the risk of capillary embolization. Unlike the contrast agents used in CT and MRI, USCAs are confined to the intravascular space, which means that after injection, they remain in the blood pool and do not diffuse into the extracellular space. The gas content is gradually eliminated from the blood through the lungs, whereas the stabilizing components are filtered by the kidneys and eliminated by the liver.

The acoustic behavior of microbubbles in a US beam is complex, and contrast-specific imaging techniques are required to achieve clinically useful enhancement. With these techniques, USCAs increase the intensity of the echo signal in gray-scale harmonic and Doppler modes by 10 to 30 dB (10 to 1,000 times) during a mean of 5 minutes, depending on the contrast agent used. Total duration of a US evaluation with contrast agent is about 20 minutes, and general anesthesia is not required.

Contrast-enhanced US improves the detection of perfusion and vascularity of organs. Lesions that are perfused differently from healthy tissues will be differentially enhanced after USCA administration. For example, regions with segmental infarction will be indicated by a so-called signal void representing the lack of a vascular supply, whereas regions with high vascularization such as malignant tumors will appear as hypechoic areas. These features also allow differentiation of malignant and benign tumors in some situations. Ultrasonographic contrast agents can also be used as blood-pool tracers (functional imaging), in an approach similar to that for scintigraphic tracers, by use of time-intensity curves.

During the last decade, several USCAs have been developed. In the United States, only 3 USCAs are available and approved by the FDA (Table 1). The purpose of this literature review was to briefly characterize the imaging techniques needed to perform a CEUS evaluation, provide veterinary practitioners with an overview of indications for CEUS as an alternative to other diagnostic methods, compare characteristics of CEUS with those of other diagnostic modalities, review some potential limiting factors for the use of USCA, and report future possibilities for CEUS. In all studies involving CEUS that are discussed herein, CHUS was used as a contrast-specific imaging technique unless otherwise mentioned.

### Contrast-Specific Imaging Techniques

Fundamental B-mode, gray-scale imaging results in poor detectability of USCA in the presence of tissue; thus, it cannot be used for CEUS examinations. All Doppler modalities are sensitive to the presence of microbubbles, but this excessive sensitivity results in color blooming (color Doppler) or flash (power Doppler) artifacts because of strong Doppler signals caused by moving tissue, which decreases the usefulness of these modalities.

Consequently, contrast-specific techniques are required to achieve clinically useful enhancement and decrease the frequency of artifacts. Several techniques have been developed such as second harmonic imaging (used in CHUS), pulse-inversion harmonic imaging, cadence-contrast pulse sequencing, and power (amplitude) modulation. On the basis of these techniques, each ultrasound machine company has developed its own contrast-specific technology.

Each one of the aforementioned contrast-specific techniques exploits the nonlinear acoustic properties of USCAs caused by asymmetric oscillations of the microbubbles within a US beam. This phenomenon results in returning echoes not only at the fundamental frequency (f₀), but also at multiples of this frequency (ie, harmonics; eg, 2f₀, 3f₀, and 4f₀). In CHUS, sound is transmitted at the fundamental frequency f₀ and the returning echoes are filtered at the second harmonic 2f₀ (highest intensity...
Liver nodules are common in dogs. In fact, focal hepatic hyperplastic nodules have been detected in dogs 6 years of age and in all dogs 14 years of age.

### Conditions in Which CEUS is Warranted

In veterinary medicine, there are only a few reports of studies in which CEUS was performed. Ultrasonographic contrast agents were mainly used to evaluate the liver and spleen in clinically normal and diseased dogs. Furthermore, CEUS has been used clinically to evaluate the kidneys, pancreas, lymph nodes, and naturally developing portosystemic shunts in dogs.

#### Examination of the Liver—Examination of focal liver lesions is the main indication of CEUS in dogs and cats. The evaluation of focal lesions with conventional, unenhanced US is based on the gray-scale morphology (analysis of differences in echogenicity from the surrounding healthy liver tissue) and Doppler information regarding macrovascular flow (hypervascularization or hypovascularization). It facilitates unambiguous diagnosis of liver cysts (anechogenic content) or calcifications (presence of an acoustic signal void); however, some more highly differentiated processes may be associated with little or no contrast enhancement of portal veins) that can start approximately 30 to 45 seconds after injection, persists for 2 minutes after injection, and reveals a blood flow peak after 13 to 60 seconds. The delayed phase (ie, enhancement of microspheres throughout the liver parenchyma) persists until the USCA is no longer detectable in the hepatic parenchyma, which may take 4 to 20 minutes, depending upon the USCA used.

#### Lesion detection—Contrast-enhanced US is useful for detecting small and ill-defined malignant lesions or isoechogenic lesions, which are often invisible via conventional US. Compared with conventional US, CEUS can detect additional lesions in 40% to 45% of human patients. The technique is particularly superior with respect to detection of metastases because such processes may be associated with little or no contrast enhancement (hypoechogenic defects or signal voids; Figure 2). This superiority was evident in a study of 3 dogs with splenic hemangiosarcoma, in which hepatic lesions did not appear in conventional US images but did appear as clearly defined nodules in CEUS images. Most hepatocellular carcinomas appear similarly as metastases in delayed-phase imaging (Figure 3); however, some more highly differentiated tumors are isoechogenic, compared with the adjacent liver, which makes their detection far more difficult.
Lesion characterization—The changes in enhancement throughout the various aforementioned vascular phases contribute to diagnosis of liver lesions.\textsuperscript{13,43,49} The arterial phase provides information on degree and pattern of vascularity, whereas the portal-venous and delayed phases provide information about the clearing of USCA from the lesion, compared with normal liver tissue.\textsuperscript{13,34,43} Most benign liver lesions appear to have typical sustained enhancement (Figure 4). This means that a lesion is enhanced to a greater degree than the liver during the arterial phase and continues to be enhanced to a degree equal to or greater than that of the liver during the portal-venous phase. Thus, benign liver lesions can be differentiated from most malignant lesions, which have early arterial phase enhancement greater than that of the liver and portal-venous and delayed phase enhancement less than that of the liver (so-called early wash-in, early washout phenomenon; Figures 2 and 3). This happens because malignant liver tumors, whether primary or secondary, obtain most of their blood supply from the hepatic artery.\textsuperscript{15,34,43,50}

In a study\textsuperscript{23} of 32 dogs with spontaneously developing liver nodules, nodules that were hypoechoic in the portal and late phase were significantly ($P < 0.001$) associated with malignancy, and the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of this diagnostic approach were high (100%, 94%,...
94%, 100%, and 97%, respectively). To this end, CEUS is a valuable alternative to invasive diagnostic procedures such as fine-needle aspiration or core biopsy.

**Examination of the spleen**—Nodular splenic disease is common in dogs and can be caused by non-neoplastic (focal nodular hyperplasia, hematoma, infarct, or abscess) or neoplastic processes (hemangiosarcoma, lymphoma, malignant histiocytosis, or other mesenchymal tumors). A conventional US examination can distinguish among hypoechoic, hyperechoic, and complex focal splenic parenchymal abnormalities but cannot help to determine the etiology of the process. Moreover, there is a considerable overlap in the US appearance of focal splenic lesions, and splenic fine-needle aspirates only yield a correct diagnosis in 61.3% of affected dogs. Splenectomy followed by histologic examination is therefore often necessary to determine the prognosis and therapeutic options.

Contrast-enhanced US of healthy spleens in dogs has revealed 2 vascular phases (Figure 5). In the early arterial phase, a nonhomogenous, patchy enhancement corresponds to variable flow rates through the cords and sinuses of the red pulp of the spleen and strongly complicates interpretation. Therefore, it is recommended that splenic assessment and lesion detection be performed during the delayed phase, at least 60 seconds after injection. During the delayed phase, the spleen becomes homogenously enhanced for about 5 to 7 minutes.

In the canine spleen, CEUS can help in the characterization of focal lesions, and hemangiosarcoma and lymphosarcoma have specific perfusion patterns. The methodology can also be used for improving the visualization of infarction, detection of microabscesses or local nodular lesions (Figure 6), and visualization of traumatic injuries (Figure 7).

**Examination of the pancreas**—In veterinary medicine, USCAs were only recently used to quantitatively assess vascular perfusion in healthy canine pancreas and to detect abnormal vascular perfusion patterns in dogs with pancreatitis. Significant differences in perfusion variables are evident between clinically normal dogs and dogs with pancreatitis.

![Figure 3](image_url)

**Figure 3**—Ultrasoundographic images of typical so-called early wash-in, early washout enhancement of the subsequent vascular enhancement phases in a 9-year-old Labrador Retriever with hepatocellular carcinoma. A—Conventional US reveals a large, clearly defined, round heterogeneous mass (arrows) surrounded by a small rim of healthy homogenous liver tissue (asterisks). B—During the early arterial phase of CEUS (contrast-tuned imaging mode), the feeding arteries (arrows) of the malignant mass are highlighted by contrast agent. The contrast agent has not yet reached the healthy liver, which is homogenously hypoechoic (asterisks). C—The subsequent portal-venous phase reveals earlier enhancement of the mass (arrows) than that of the surrounding healthy liver parenchyma (asterisks). D—The opposite occurs during late phase of enhancement, in which the decrease in enhancement of the mass (arrows) precedes the surrounding liver parenchyma (asterisk). These features are characteristic of a neoplastic process.
In humans, chronic mass-forming pancreatitis and pancreatic carcinoma have the same conventional US appearance and clinical symptoms. When USCAs are used, these 2 entities can be differentiated with an overall accuracy of 96% on the basis of differences in the intralesional parenchymographic phase. This phenomenon also results in a differential uptake of contrast agent by pancreatic carcinomas and endocrine tumors such as insulinomas. Endocrine tumors appear hypervascular in contrast to carcinomas. These features allowed the authors to diagnose a carcinoma in 1 dog and an insulinoma in another (both of which were histologically confirmed) by means of a contrast-specific imaging technique that uses a low-frequency transducer and sulfur hexafluoride microbubbles as a contrast agent.

**Examination of kidneys**—In evaluations of kidneys of human patients, dogs, and rabbits, USCAs yield more accurate morphologic and quantitative information about cortical vascularity than does power Doppler US. In healthy kidneys, use of USCAs results in early enhancement of the renal arteries during the arterial phase of vascular enhancement and subsequent homogenous enhancement of the renal cortical parenchyma and medulla, which become isoechic with respect to the renal cortex (Figure 8). Vascular perfusion in healthy canine kidneys has been ultrasonographically evaluated with the aid of perflutren lipid microspheres. Maximum enhancement of the renal cortex occurs at 15 seconds and of the renal medulla at 30 seconds after injection of contrast agent. The authors have used CEUS in the detection and characterization of focal renal mass–like lesions in 6 dogs (2 renal cell carcinomas, 2 hemangiosarcoma metastases, 1 simple renal cyst, and 1 hematoma) by use of an aforementioned contrast-specific imaging technique. Therefore, we believe CEUS can potentially aid in the discrimination between primary and secondary malignant renal lesions and increase the ability to detect lesions, compared with conventional US. It also improves the detection of fluid-filled cavities.

![Figure 4](image1)

**Figure 4**—Ultrasonographic images of the liver of a dog with hyperadrenocorticism. **A**—In a conventional US image, hepatomegaly is evident, and the liver parenchyma is hyperechogenic. However, additional small, ill-defined hyperechoic areas (open arrows) are evident. **B**—During the late phase of enhancement, CEUS (contrast-tuned imaging mode) reveals a diffusely homogenous liver parenchyma indicating the benign nature of the hyperechoic lesions.

![Figure 5](image2)

**Figure 5**—Contrast-enhanced US images (contrast-tuned imaging mode) of a healthy canine spleen. The image from the arterial phase (**A**; 20 seconds after injection of contrast agent) reveals nonhomogenous enhancement (arrows) of the splenic parenchyma, followed by subsequent homogenous enhancement starting at approximately 50 seconds after contrast agent administration (**B**). This pattern suggests assessment of the spleen and its lesions should begin at 60 seconds to avoid misdiagnosis of hypoechogenic lesions as malignant. Notice the presence of a moderate amount of free abdominal fluid caused by diffuse liver disease (asterisk).
Examination of lymph nodes—Several changes evaluated by means of US can help in discriminating between healthy and diseased (inflamed, lymphomatous, or metastatic neoplastic) lymph nodes. The combination of lymph node size, distribution of vascular flow, and pulsatility index is reportedly the most accurate means of differentiating among these 4 diagnostic categories; the associated classification error is 23%. When only 2 diagnostic categories are used (benign or malignant), that error is reduced to 11%.

Measurement of blood flow with Doppler modalities is possible in more than half of healthy lymph nodes and in slightly more than 80% of reactive and malignant nodes. The insensitivity is caused by a lack of detector sensitivity. In humans, signal enhancement with USCs remarkably improves the sensitivity and provides better conspicuity of vessel morphology in all lymph nodes (2.13 times as many vessels are detectable via CEUS vs power Doppler US).

Contrast-enhanced harmonic and power Doppler US have been used to characterize peripheral lymph nodes in dogs with lymphoma. In 81.8% of those dogs, fundamental US resulted in loss of the so-called hilus sign. However, CHUS with perfluorin lipid microspheres as a USCA resulted in increased detection of the fine angioarchitecture of the lymph nodes, compared with the detection ability of power Doppler US. Hence, CHUS resulted in a more accurate characterization of affected lymph nodes. In the same study of 11 canine peripheral lymph nodes evaluated via CHUS,
45% had displacement of the central hilar vessels, 45% had aberrant vessels, 64% had pericapsular vessels, and 36% had subcapsular vessels.

When staging cancer, a finding of lymph node metastases is an important prognostic determinant and guides proper choice of treatment. The sentinel lymph node is defined as the first node to receive lymphatic drainage from a neoplasm. Radioactive technetium scintigraphy or injection of blue dye into or near a tumor is used to detect a sentinel lymph node. In dogs injected SC with a USCA, microbubbles can yield contrast-enhanced power Doppler signals in 85% of the sentinel lymph nodes. When smaller bubbles are used, the percentage of signals in the lymph node increases to 94%. It must be emphasized that these findings are specific to superficial lymph nodes, and additional studies are needed to assess the behavior of USCAs in abdominal lymph nodes.

Vascular application—Initial results achieved when CHUS was used in 3 dogs with congenital extrahepatic portosystemic shunts indicated shorter times to peak perfusion in the liver (7.0 ± 2.0 seconds), compared with results in clinically normal dogs (22.8 ± 6.8 seconds). This finding suggests increased hepatic arterial blood flow in dogs with shunts, compared with those without shunts, and supports the diagnosis of a shunt. However, CEUS was not able to increase the visibility of the shunt vessel.

Quantification of USCAs via time-intensity curves—Quantification of USCAs is of fundamental importance because it allows objective evaluation of the degree of tissue perfusion and detection of diffuse tissue changes. A USCA can be used as a tracer for dynamic evaluations of organs such as the liver, kidney, or brain. In addition, the arteriovenous transit time of a USCA can be digitally processed, and the resulting time-intensity curves will allow measurement of the rate of uptake and clearance of contrast agent in a specific location.

In the past few years, several sophisticated US systems have been developed to include a built-in software package that generates time-intensity curves. Compared with US units that use external software, such systems result in a reduction in errors. This is attributable to a decrease in the electronic transfer of the images between various machines, which causes loss of image quality. Furthermore, the measurement system is standardized. With these software systems, ROIs can be drawn to highlight vessels or areas in the adjacent parenchyma. For each ROI, the gray-scale or Doppler signal changes can be calculated during a certain duration and displayed as a time-intensity curve. Mathematic analysis of these curves yields quantitative hemodynamic indices relating to blood flow within volumes of tissue or within single vessels.

After IV bolus injection of a USCA, a time-intensity curve will have a characteristic dilution shape consisting of a 2-phase dose response (Figure 9). The first phase is indicated as a rapid rise in intensity followed by a rapid linear decrease during an early phase in which the contrast agent is eliminated (so-called washout phase). The phase 1 kinetics correspond to the first pass of contrast agent through the arterial circulation and do not depend on cardiac output, dose of contrast agent administered, and size of the ROI. The second phase is indicated by a slow linear decrease of the washout...
Lesions measuring up to 5 mm are clearly detected via CEUS, and even lesions <3 mm can be detected. 78,81,83,84

Unlike CECT and CEMRI, CEUS permits real-time analysis of tumor perfusion, which provides better temporal resolution, and does not require that subjects be anesthetized. 53,85 In addition, compared with CECT and CEMRI, CEUS is more cost-effective, can be performed faster, and does not involve ionizing radiation. As such, CEUS represents a competitive alternative to the established imaging methods. 34,36,79,81

Compared with cytologic evaluation of fine-needle aspirates or histologic examination of tissues, CEUS has higher specificity. 81,84 and is also noninvasive and relatively easy to perform and discriminates well between benign and malignant processes. 27

Figure 9—Time-intensity curves of the administration of contrast agent into a canine liver via a bolus injection (A) and an infusion (B) as detected via CEUS. After injection of a bolus of contrast agent, a typical wash-in–washout curve is obtained. There is a 2-phase dose response. A sharp rise in intensity is followed by a more gradual decrease during the washout phase. After infusion of contrast agent, enhancement is progressive with a subsequent plateau that persists until the end of the infusion.

Phase and is the predominant effect detected after IV injection in terms of duration. The kinetics of phase 2 represent the recirculation of contrast agent between venous circulation. These kinetics depend on the elimination of contrast agent from the blood pool, dose of contrast agent administered, and size of the ROI but not on cardiac output. The first phase is much shorter than the second phase. 74,78 In sequences associated with constant rate infusions, the time-intensity curves will indicate progressive enhancement with a plateau-like enhancement profile that persists until infusion is concluded. 70,71 Changes in vascularity and blood flow secondary to pathologic processes are represented in the time-intensity curve as alterations of its shape. 14,67

Time-intensity perfusion curves have been generated for the liver, 25,47,72 spleen, 26 pancreas, 7 and kidneys of clinically normal dogs. Choice of anesthetic protocol in dogs with healthy livers can influence perfusion indices from time-intensity curves. 43

Comparison of CEUS With Other Diagnostic Modalities

In human medicine, CEUS (in combination with Doppler US) is equivalent to dynamic computed tomography and superior (higher sensitivity and specificity) to Doppler US for the diagnosis of blood flow abnormalities in patients with macrovascular diseases (stenosis, thrombosis, or abnormal portal collateral vessels) of the portal venous system, kidney, and liver. 67,12,77,78 Contrast-enhanced US is also superior to CECT and equivalent to CEMRI in characterization of focal liver lesions. 15,49,79,80 Detection of liver metastases with CEUS is as sensitive and accurate as detection with CECT, if not more so. 14,46,81,82 Lesions measuring up to 5 mm are clearly revealed via CEUS, and even lesions <3 mm can be detected. 78,81,83,84

Safety factors—in general, USCAs are well tolerated for abdominal examinations, with few adverse reactions reported. In humans, reported adverse events are typically minor (eg, headache, nausea, altered taste, or sensation of heat) and self-resolving. 4,86 In Europe, USCAs are widely used in human medicine without particular restriction. In the United States, however, USCA use in humans is highly regulated because of the potential for serious cardiopulmonary reactions. In October 2007, the FDA issued a warning on the use of perfluorocarbon-based USCAs (eg, perflutren lipid microspheres and perflutren protein–type A microspheres) for echocardiography and stated that the use of such products in humans with unstable cardiopulmonary status is contraindicated. 87 Use of USCAs in veterinary medicine has not been approved by the FDA. Therefore, use of USCAs in animals is strictly extra-label. To the authors’ knowledge, no adverse reactions in dogs have been reported.

Stability and price of USCAs—once reconstituted, a USCA remains stable only for several hours, depending on the agent used (maximum of 6 hours for sulfur hexafluoride and 12 hours for perflutren lipid microspheres). This means that a new vial of contrast agent must be used for each US subject, which substantially increases the cost for pet owners. In the United States, 1 vial of contrast agent costs approximately $150. This limitation can be overcome by scheduling several subjects within the same period so that 1 vial can be used, resulting in less wastage and reduced cost.

Need for adequate equipment—Performance of CEUS requires sophisticated US machines, which must be equipped with Doppler modalities, special low-frequency transducers, software that permits performance of contrast-specific imaging, and, eventually, software for quantitative evaluation of CEUS images. The dependence on such machines also adds to the cost of CEUS.
**Future Possibilities for CEUS**

**Targeted (molecular) imaging**—Because their size prevents extravasation, the currently used USCs are in fact passively (nonspecifically) targeted to the blood pool. Some microbubbles, which are as yet unavailable in the United States, are able to preferentially accumulate in tissues such as the liver (NC100100,¹³ SHU563A,¹³ and SHU508A),¹³,¹⁴,¹⁸ spleen (sulfur hexafluoride),¹⁸ or activated endothelium. The mechanisms of this nonspecific targeting are poorly understood but likely depend on the properties of the shell and resting diameter of the contrast agent.¹³¹⁸ Novel tissue-specific (active targeting) USCs have recently been developed, and these agents can improve the assessment of certain organs by increasing the contrast resolution attributable to preferential uptake by the organs.⁰⁰⁰¹

Several strategies have been used to target USCs to diseased areas. The most common method of active targeting is incorporation of bioactive adhesion molecules (antibodies, peptides, or other ligands), which recognize disease-related antigens (receptors), into microbubble shells, resulting in so-called targeted USCs.¹³⁷⁸ Of the many available molecular markers, those used in targeted US have so far been limited to markers administered via IV injection.¹¹ Because microbubbles are intravascular agents, the assessed disease processes must be characterized by antigens that are expressed within the vascular compartment.¹¹ Therefore, these targeted USCs can be used to highlight inflammation (by conjugating ligands [monoclonal antibodies] to their surface, which then bind to endothelial cell adhesion molecules [ICAM-1, P-selectin, or VCAM-1]), activated IV thrombi (via USCs that bind to glycoprotein IIb/IIIa receptors, which are abundant at the surface of activated platelets), tumors (neoplastic endothelial cells overexpress integrins αβ₃ and αβ₅, which can bind to polymeric microbubbles conjugated with a generic peptide sequence arginine-glycine-aspartatic acid), and lymph nodes (via antibody on microbubble surface that targets the L-selectin ligand expressed in lymph node venules).¹¹⁸⁹⁰⁰⁰¬⁹⁴

**Therapeutic applications**—Other concepts that are being explored include targeted drug delivery that makes use of the aforementioned targeted microbubbles. Because of their special acoustic and biological properties, microbubbles can be very promising as a vehicle for drug and gene delivery. The most important disadvantages of CEUS include poor stability of USCs and the need for specialized equipment, which increase the cost of this imaging technique. Moreover, in humans, some adverse effects associated with administration of USCs have been reported.

**Conclusion**

Contrast-enhanced US can be used in dogs to increase the intensity of the bloodpool echo signal. Its main indication is the assessment of focal lesions in the liver and spleen, particularly for differentiation between benign and malignant processes. Evidence suggests that CEUS is a valuable alternative to invasive diagnostic procedures such as fine-needle aspiration or core biopsy. The diagnostic method allows real-time analysis of tumor perfusion, does not require that subjects be anesthetized, has no ionizing radiation, can be performed quickly, and is more accurate, compared with fine-needle aspiration or core biopsy. As promising results in human medicine have suggested, USCs can also be useful as vehicles for drug and gene delivery. The most important disadvantages of CEUS include poor stability of USCs and the need for specialized equipment, which increase the cost of this imaging technique. Moreover, in humans, some adverse effects associated with administration of USCs have been reported.

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