Contrast-Enhanced Ultrasonography for Characterization of Liver Nodules

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Detection and characterization of liver tumors represents a diagnostic imaging challenge in both human and veterinary medicine.

Routine gray-scale B-mode ultrasonography is sensitive for detecting focal disease in the liver but is nonspecific. It is not possible to discriminate between benign lesions and malignancies in most instances because both may have similar echo patterns.[1]

The purpose of this study was to:

- Assess the sensitivity, specificity, and accuracy of contrast-enhanced ultrasonography (CEUS) using a second-generation agent containing perfluoropropane (Definity, bms.com) for differentiation between benign and malignant liver lesions in dogs

- Describe and compare the CEUS vascular morphology and enhancement patterns of primary and metastatic lesions.

STUDY OVERVIEW

- This study aimed to assess the vascular morphology and phases of enhancement of hepatic nodules using a perfluoropropane ultrasound contrast agent to differentiate malignant from benign lesions.

- Benign lesions were isoechoic with the surrounding liver during the portal phase in all but 2 of 15 lesions.

- Malignant nodules were hypoechoic to surrounding liver parenchyma during the portal phase on CEUS in all but 1 dog. In addition, all malignant nodules showed intralesional vessels during the portal phase.

- A smaller number of metastatic lesions had additional peripheral feeding vessels evident during the arterial phase that were not present in primary neoplasms. These features were absent in all benign lesions except for 1 diagnosed as necrotic.

- The presence of necrosis within a liver nodule may pose difficulty for the differentiation of benign from malignant nodules.

- All hepatic masses diagnosed as hepatocellular carcinoma were hypoechoic to surrounding liver parenchyma during the portal phase. However, assessment of large masses with CEUS may not be indicated because of their tendency to have necrotic centers that would appear as regions of hypoperfusion and, therefore, could be misdiagnosed as malignant. Necrotic lesions have absent or poor vascular supply and in this study were hypoechoic in the arterial phase, which was nondiscriminatory between benign and malignant lesions.[2]

- The results of this study are in accordance with previous studies in which CEUS showed high sensitivity and specificity to characterize hepatic nodules.[3-6]

STUDY PARTICIPANTS

The 30 dogs included in this study (mean age, 11 years; range, 5–15 years) had been presented for routine abdominal ultrasound. They were selected if at least 1 nodule was detected in the liver during ultrasound examination and if a definitive diagnosis was obtained by histopathology or cytology of the liver nodules. Dogs with hepatic masses (lesions > 3 cm) were included in the study if hepatic nodules were also present. In these dogs CEUS was performed to evaluate the nodules.

IMAGING STUDY

Two-dimensional gray-scale and CEUS examinations were performed. The region of the liver containing nodules was identified and the probe was maintained in the same location for the CEUS examination.

An IV bolus injection of the contrast agent was administered. Based on a previous study, dogs weighing < 20 kg received 0.1 mL of contrast medium; those weighing ≥ 20 kg received 0.2 mL.[7] Two milliliters of saline were administered immediately after each injection. No complications were noted after administration of contrast.

A 1-minute duration cine loop was recorded during contrast enhancement and still images and clips were digitally stored and transferred to a picture archive communication system (PACS) (soundeklin.com). Ultrasound-guided fine-needle aspiration (FNA) or biopsy of the nodules was performed using a 22-gauge, 1.5-inch needle attached to a 3- or 5-mL syringe for FNA and an 18-gauge Tru-cut biopsy needle for core tissue samples. No complications occurred after these procedures.

EVALUATION & STATISTICAL ANALYSIS

For each study, the echogenicity of the normal liver parenchyma and the nodules (after administration of contrast medium) were subjectively assessed for 1 minute. In both the arterial and portal phases the nodules were evaluated for presence or absence of

Table 1. Diagnosis of Hepatic Nodules

Liver Nodules	Diagnosis		Agreement with CEUS	
	Cytology	Histopa-thology	Yes	No
Benign				
Lymphoid infiltration	1		1	
Necrosis	1			1
Nodular hyperplasia		1	1	
Normal	1		1	
Steroid hepatopathy		1	1	
Vacuolar hepatopathy	7	3	9	1*
Total	**10**	**5**	**13**	**2**
Malignant				
Hemangiosarcoma	4	1	5	
Hepatocellular carcinoma	2	4	5	1**
Histiocytic sarcoma	2		2	
Insulinoma	1		1	
Plasmacytoma	1		1	
Total	**10**	**5**	**14**	**1**

*Diagnosed with cytology; ** diagnosed with histopathology
CEUS = contrast-enhanced ultrasonography*

vessels. The distribution of vessels (when present) was classified into 3 patterns (**Figure 1**):
- Feeding vessels
- Perilesional vessel (ring enhancement)
- Intralesional vessels.

The lesions were classified as likely to be malignant if they appeared hypoechoic to surrounding liver parenchyma and benign if they appeared iso- or hyperechoic to liver parenchyma in the portal venous phase (30–60 seconds).[8]

The sensitivity, specificity, and accuracy of CEUS to differentiate malignant from benign lesions was calculated. A contingency table analysis was performed using software with a 2-tailed Fisher's exact test to compare the proportional distributions using a level of significance of $p < 0.05$.

STUDY RESULTS

Of the 30 dogs in this study, 15 were diagnosed with benign nodules and 15 with malignant nodules. The diagnosis was based on histopathology in 10 cases and cytology in 20 cases. The diagnosis and agreement with CEUS are summarized in **Table 1**.
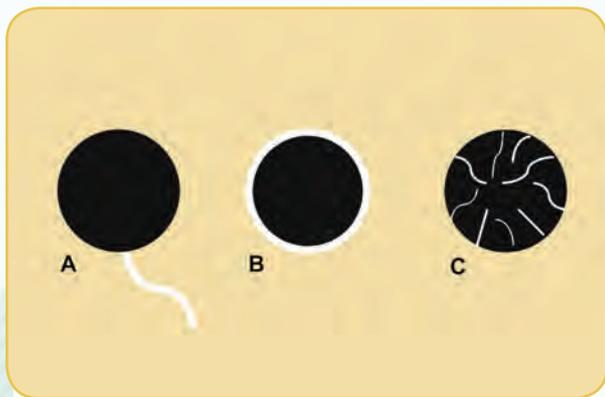

Figure 1. Diagram showing the classification pattern of vessels: feeding vessel (A), perilesional vessel or ring-enhancement pattern (B), and intralesional vessels (C)

PREVIOUS STUDIES

CEUS of normal canine livers has been previously described and its use for detection and characterization of liver nodules in dogs is being reported with increasing frequency. However, only 2 studies describe the use of an agent containing perfluoropropane and only 1 study included primary liver tumors.[3,4,7-10] Three studies describe CEUS and the use of different types of contrast medium for characterization of liver lesions in dogs.[4,9,10] Vascular morphology during the arterial and portal phases had not been described for hepatic nodules in dogs when using CEUS.

Table 2. CEUS Pattern of Nodules in Dogs with Benign Lesions

Lesion Type	Portal Phase	
	No vessels; homogenous enhancement	Intralesional vessels; hypoperfusion
Lymphoid infiltration	1	
Necrosis		1*
Nodular hyperplasia	1	
Normal	1	
Steroid hepatopathy	1	
Vacuolar hepatopathy	9	1*

* Diagnosed as malignant by CEUS
CEUS = contrast-enhanced ultrasonography

Two-Dimensional Gray-Scale Ultrasonography

The appearance of livers with benign nodules on 2-dimensional gray-scale ultrasound varied widely:

- Liver size ranged from normal to severely enlarged with rounded margins.
- Hypoechoic, hyperechoic, or mixed echogenic nodules were present.
- Target lesions were found in 2 dogs and multiple cystic lesions in 1.
- Three dogs had a complex mass ranging in size from 5 to 10 cm in addition to multiple nodules. Of these, 2 had a diagnosis of hepatocellular carcinoma and 1 of hemangiosarcoma.

Contrast-Enhanced Ultrasonography

After administration of contrast:

- Thirteen of the 15 dogs with benign lesions had homogeneous enhancement of the liver, with the nodules being isoechoic to liver parenchyma during arterial and portal venous phases, which impaired the visualization of the nodules (**Table 2**).
- In 1 dog with multiple cystic lesions, 1 nodule remained hypoechoic during arterial and portal phases with multiple small intralesional vessels in the portal phase. This nodule was diagnosed as malignant by CEUS, but was diagnosed as necrosis based on cytology. There was no enhancement of the cysts after contrast administration.
- One dog with a cytologic diagnosis of vacuolar hepatopathy had nodules that remained hypoechoic compared to the surrounding liver parenchyma during the arterial and portal phases and had multiple small intralesional vessels in the portal phase. These nodules were diagnosed as malignant based on CEUS.

EQUIPMENT & USE

- Ultrasound machines equipped with contrast harmonic imaging software and transducers (iu22, philips.com; MyLab 70 XVG, esaote.com) were used.
- Gray-scale ultrasound was performed using a convex 8-5 MHz transducer.
- CEUS was performed using a convex 5-2 MHz or a linear 9-3 MHz contrast harmonic transducer (based on size of dog). The mechanical index was maintained at 0.14 to minimize microbubble destruction.
- Contrast medium was administered manually via an IV catheter placed in the cephalic vein.

- In all 3 dogs with a complex mass the additional nodules appeared homogenous to the surrounding liver parenchyma after contrast administration; they were correctly diagnosed as benign by CEUS. The final diagnosis of the additional nodules was nodular hyperplasia (1 dog) and vacuolar hepatopathy (2 dogs). All 3 masses had a final diagnosis of hepatocellular carcinoma. During CEUS, these masses showed multiple areas of hypoperfusion with intralesional vessels.

Malignant Nodules

With CEUS, 14 of 15 dogs with malignant nodules had a statistically significant (p = 0.0001) different pattern of enhancement than that of dogs with benign lesions (**Table 2**).

- In all of these dogs the nodules appeared hypoechoic to surrounding liver parenchyma and had multiple small intralesional vessels in the portal phase (**Figure 2**).
- On the arterial phase, early ring enhancement, followed by a hyperechoic appearance compared to surrounding liver parenchyma and feeding vessels, was seen in 2 metastatic tumors, insulinoma, and histiocytic sarcoma (**Figure 3**). The other histiocytic sarcoma also exhibited pronounced hyperechogenicity during the arterial phase.
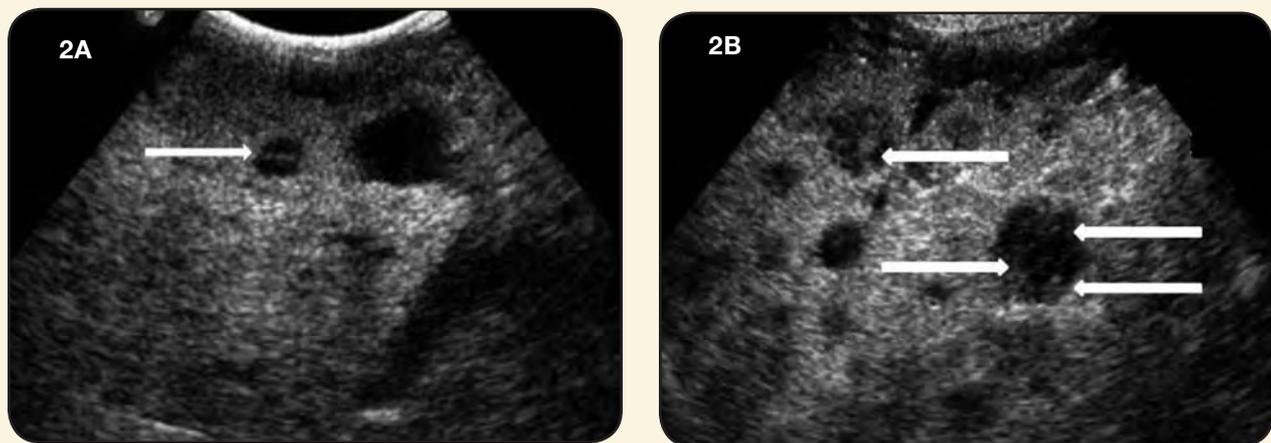

Figure 2. (*A*) Portal phase of CEUS of a dog with final diagnosis of hepatocellular carcinoma showing small intralesional vessels (arrow). The nodules appeared hypoperfused compared to the surrounding liver parenchyma during this phase and were diagnosed as malignant with CEUS. (*B*) Portal phase of CEUS of another dog with hepatocellular carcinoma showing small intralesional vessels (arrows). The nodules appear hypoperfused compared to the surrounding liver parenchyma during this phase and were diagnosed as malignant.
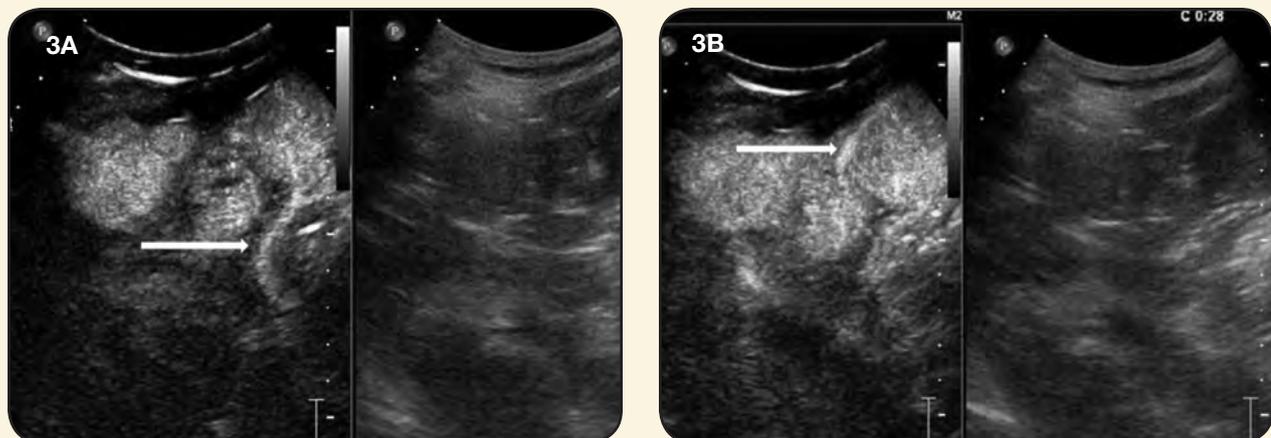

Figure 3. (*A*) CEUS image (left) in the arterial phase showing hyperperfusion and a feeding vessel (arrow) of a nodule diagnosed as histiocytic sarcoma and corresponding gray-scale image (right). (*B*) CEUS image (left) in the arterial phase of the same lesion as shown in Figure 3A and corresponding gray-scale image (right). Note the perilesional vessel creating a ring-enhancement pattern (arrow). The nodule appeared hypoperfused compared to the surrounding liver parenchyma on the portal phase of CEUS.

- These patterns were different than those of primary neoplastic lesions, which exhibited no ring enhancement or hyperechogenicity during the arterial phase.
- In 1 dog with a complex hepatic mass due to metastatic hepatocellular carcinoma on histopathology, the hepatic nodules appeared homogenous to the surrounding liver parenchyma in both arterial and portal venous phases and were not visualized after contrast administration, resulting in being diagnosed as benign by CEUS.

DISCUSSION

By increasing the intensity of the signal from blood, intravascular contrast media help display the morphology and shape distortions of tumor vasculature, which allows mapping and assessment of the characteristics of vessel flow.[11]

The main difference between benign and malignant lesions is:

- Benign lesions, except cysts and thrombosed

EVERYDAY APPLICATION

This study shows the importance of ultrasound image interpretation of liver nodules in general practice. Hepatic nodules are a very common finding, especially in older canine patients. Because not all of them are malignant, tissue sampling with FNA or Tru-cut biopsy is necessary and generally safe. Alternatively, CEUS is a safe and noninvasive procedure. However, it does require specialized equipment and ultrasound probes as well as contrast medium.

hemangiomas, exhibit iso-enhancement or slight hyperenhancement compared to surrounding liver tissue during the portal phase.
- Malignant liver nodules exhibit hypoperfusion or early wash-out in the portal phase because the vascular supply of malignant tumors is provided exclusively by arterial vessels and there is no portal venous supply.[1]

Patterns of contrast-enhancement during ultrasound for specific hepatic lesions have not been extensively described in veterinary medicine. Two previous studies in dogs with hepatocellular carcinoma described contrast-enhancement patterns similar to that found in this study, with lesions remaining hypoechoic compared to liver parenchyma during the portal phase, which markedly increased the conspicuity of these lesions.[4,6] One recent study, which used a new contrast agent, described a rim pattern of enhancement with malignant histiocytosis.[9]

This study described the enhancement patterns of 3 different metastatic tumors. All exhibited hyperechogenicity during the arterial phase, with 2 (histiocytic sarcoma and insulinoma) showing ring enhancement, and all were hypoperfused compared to liver parenchyma in the portal phase, a pattern previously described for metastasis in humans and dogs.[4,6,12]

Limitations

This study's limitations were:

- The clinical diagnosis was based on cytology in most cases. The use of cytology may be problematic if more than 1 condition is present in the liver or an individual nodule. For example, the cytologic diagnosis of necrosis does not exclude the possibility that malignant neoplasia is also present.
- The number of dogs with each specific tumor type was small.

Future studies that include a larger number of dogs with primary tumors and metastasis from tumors other than hepatocellular carcinoma are needed to (1) better assess the pattern of enhancement of specific tumor types and (2) assess whether primary lesions can be differentiated from metastatic lesions based on CEUS.

Conclusion

Using enhancement patterns and vascular morphology, CEUS with perfluoropropane contrast medium can be used to differentiate malignant from benign hepatic nodules seen on gray-scale ultrasound. ∎

CEUS = contrast-enhanced ultrasonography;
FNA = fine-needle aspiration;
PACS = picture archive communication system

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