Contrast harmonic ultrasonography of splenic masses and associated liver nodules in dogs

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**Objective**—To determine whether contrast harmonic ultrasonography (CHUS) can be used in dogs to distinguish splenic hemangiosarcoma from hematoma and to accurately detect and characterize liver nodules.

**Design**—Cross-sectional study.

**Animals**—20 dogs with a splenic mass.

**Procedures**—Routine abdominal ultrasonography was followed by CHUS of hepatic and splenic lesions. Qualitative evaluation included location, enhancement pattern, and vascularity of lesions. Quantitative evaluation included peak mean pixel intensity, interval to peak intensity, area under the curve (spleen), and liver-to-lesion intensity ratio (liver). Histologic findings were compared with CHUS lesion characteristics.

**Results**—Histologic evaluation of the spleen was performed in 19 dogs, resulting in diagnoses of hemangiosarcoma (n = 11), hematoma (7), and undifferentiated sarcoma (1). Benign and malignant processes in the spleen were indistinguishable via CHUS. Histologic evaluation of the liver was performed in 18 dogs, resulting in a diagnosis of hemangiosarcoma in 5 dogs. None of the dogs with splenic hematomas had evidence of hepatic lesions by means of conventional or contrast ultrasonography, and none had histologic evidence of liver metastases. In 3 of 18 dogs, isoechoic liver nodules were detected and all were histologically benign. Five dogs had liver nodules that remained hypoechoic after contrast agent was injected; all had histologic evidence of metastatic hemangiosarcoma. Results of CHUS were used to characterize hepatic metastases with 100% sensitivity and specificity.

**Conclusions and Clinical Relevance**—Contrast harmonic ultrasonography was a noninvasive and accurate means of differentiating metastatic versus benign hepatic disease in dogs with splenic hemangiosarcoma but was not useful in distinguishing splenic hemangiosarcoma from hematoma. (J Am Vet Med Assoc 2009;234:88–94)

Contrast harmonic ultrasonography is also useful for distinguishing benign hepatic nodules from metastatic lesions and approaches, rivals, or even surpasses the

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diagnostic usefulness of cross-sectional imaging in evaluating focal hepatic lesions. In veterinary medicine, use of contrast ultrasonography has yielded similar results. The objectives of the study reported here were to use CHUS to characterize splenic and hepatic lesions in dogs with suspected hemangiosarcoma and to compare CHUS findings with histologic results. We hypothesized that CHUS would be able to distinguish splenic hemangiosarcoma from hematoma and correctly characterize liver nodules as malignant or benign.

Materials and Methods

Animals—Dogs evaluated at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania for a splenic mass suspected to be hemangiosarcoma with or without hemoabdomen, whose owners consented to histologic evaluation of their dog’s liver and spleen, were included in the study. Owner consent was obtained prior to ultrasonographic examination and contrast agent administration. The protocol used in this study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Ultrasonography—A standard abdominal ultrasonographic evaluation was performed in all dogs with an ultrasound imaging system. The precise appearance and location of all hepatic and splenic lesions were recorded. This was followed by CHUS. Software developed for use with contrast media and the same ultrasound system was used in conjunction with a 3.5-MHz curvilinear transducer, phase inversion harmonic imaging, and a low mechanical index setting (0.2, with 5% acoustic output). A bolus of a perfluoropropane lipid microsphere contrast agent was administered directly into an indwelling peripheral venous catheter at a dose of 0.1 to 0.2 mL/injection, depending on the body weight of the dog (0.1 mL when < 16 kg [35.2 lb] and 0.2 mL when ≥ 16 kg). A cephalic venous catheter was used for administration of contrast agent in all dogs except 2, in which a lateral saphenous venous catheter was used. Catheters were flushed with saline (0.9% NaCl) solution immediately after each injection, and the hepatic parenchyma and splenic lesions were evaluated independently (ie, by use of separate injections). A total of 3 or 4 injections was administered (1 or 2 injections/organ), depending on the quality of enhancement detected with each injection.

The image sequence was recorded on videotape. Scan planes were chosen in areas with representative nodules or masses detected before contrast agent was administered or, when none was detected (in the liver), in a plane in which a large portion of the parenchyma could be visualized. After imaging the chosen scan plane for 90 seconds for quantitative analysis, the transducer was swept through the parenchyma to qualitatively evaluate lesions outside the chosen scan plane and to search for previously undetected lesions. This approach was chosen to be able to evaluate most of the liver parenchyma with only 1 to 2 injections of contrast agent. Lesion size and anatomic location were evaluated and recorded as a written description and as images. The information was then conveyed to the surgeon performing the splenectomy and liver biopsy or to the pathologist performing the necropsy in an attempt to obtain histologic evaluation of the same nodules whenever possible. Surgeons and pathologists were also encouraged to biopsy any other lesion they would have typically evaluated. Sedation was not used in any dog for the purpose of ultrasonographic evaluation.

Qualitative image analysis—Subjective evaluation of the spleen involved characterization of the extent and pattern of vascularization of the splenic mass. When multiple masses were evident, the largest was chosen for analysis during administration of contrast agent.

Subjective evaluation of the liver was performed as described elsewhere. The enhancement pattern relative to the surrounding parenchyma over time was described; increased, decreased, or unchanged echogenicity and conspicuity were primarily evaluated. Liver nodules that became isoechoic with the liver parenchyma after enhancement with contrast agent were considered benign, and nodules that remained or became hypoechoic, compared with the liver parenchyma, were considered metastatic in accordance with criteria reported in the veterinary literature. With histologic evaluation used as the gold standard, CHUS findings were compared with histologic results and sensitivity, specificity, and accuracy of CHUS as a diagnostic method were calculated.

Quantitative image analysis—Videotaped image sequences were digitized frame by frame by use of a digitizer connected to a standard personal computer and stored in an uncompressed format. On the digitized image sequences, MPI over time was analyzed within an ROI by means of custom-made computer software described elsewhere. For the spleen, a single large, circular ROI was centered over the largest splenic mass, and a numeric value representing MPI was determined for each ROI over 90 seconds (at 30 frames/s). Peak MPI, interval to peak intensity (from injection), and area under the curve were determined.

For the liver, a circular ROI was drawn within an area of sonographically normal liver parenchyma and when nodules were evident, within a nodule. Frames in which respiratory movement caused only partial overlap of the ROI with the nodule were eliminated prior to data analysis. For each ROI, the MPI per second was determined. From these data, peak MPI and interval to peak intensity were estimated. In addition, the ratio between the MPI of ultrasonographically normal liver and a nodule (liver-to-lesion intensity ratio) was calculated at the time of peak normal liver MPI.

Histologic analysis—Hepatic and splenic tissues obtained via exploratory laparotomy and surgical biopsy, necropsy, or both were submitted for histologic analysis. In 1 dog that was euthanized during surgery, the spleen was inadvertently not submitted for histologic analysis, and the splenic
data from that dog were excluded from the study. In a second dog, a liver biopsy was inadvertently not performed. In a third dog, a liver lesion detected ultrasonographically was clearly identified during surgery but the primary surgeon elected not to biopsy the lesion because of risk of hemorrhage and instead submitted a tissue sample from a different liver lobe. The liver data from those 2 dogs were also excluded from the study.

A diagnosis of hemangiosarcoma was made when neoplastic cells were evident and appeared to form recognizable vascular channels within the tumor. A diagnosis of hematoma was made when there were collections of blood within the tissue, particularly when the blood collected in areas of lymphoid follicular hyperplasia with lymphoid aggregates.

**Statistical analysis**—Peak MPI, interval to peak intensity (from injection), and area under the curve for splenic hematoma and splenic hemangiosarcoma were compared by use of an unpaired 2-tailed t test. Peak MPI and interval to peak intensity of ultrasonographically normal liver, benign liver nodules, and metastatic liver nodules were evaluated with a 1-way ANOVA. Post hoc evaluation for individual differences was performed by use of the Fisher least significant difference method. The liver-to-lesion intensity ratio for benign nodules and hemangiosarcoma nodules was analyzed by means of a nonparametric Mann-Whitney U test. Statistical significance was defined as \( P < 0.05 \). Values are reported as means and 95% CIs. All statistical analyses were performed by use of a commercial statistical software package.

**Results**

**Animals**—Twenty dogs were included in this study. There were 11 castrated males, 8 spayed females, and 1 sexually intact female. Mean age of the dogs was 10.6 years (95% CI, 8 to 13 years), and mean body weight was 27.8 kg (61.2 lb; 95% CI, 6.6 to 51 kg [13.2 to 112.2 lb]). In addition to 5 mixed-breed dogs, breeds represented included Labrador Retriever (n = 4), Golden Retriever (2), Soft Coated Wheaten Terrier (2), and one of each of the following: Airedale, Beagle, Schipperke, Pug, Cocker Spaniel, American Pit Bull Terrier, and Rhodesian Ridgeback. Eighteen of 20 (90%) dogs underwent exploratory laparotomy and splenectomy; 2 (10%) dogs were immediately euthanatized and underwent necropsy.

**Conventional ultrasonography**—A final diagnosis for the spleen was available for 19 dogs. Conventional ultrasonography revealed that all 19 dogs had ≥ 1 splenic mass. Nine of 19 dogs had concurrent liver...
nodules, other liver abnormalities (eg, mottled echotexture or hypoechoic or hyperechoic parenchyma), or both. Splenic hemangiosarcoma was diagnosed in 11 of 19 spleens (Figure 1); undifferentiated sarcoma was diagnosed in 1 other spleen. Benign splenic lesions were detected in 7 of 19 dogs. Of these 7 dogs, 5 had splenic hematomas and 2 had ≥ 1 splenic hematoma with hyperplasia (Figure 2).

Results of histologic examination of liver specimens were available for 18 dogs. Hemangiosarcoma was diagnosed in 5 dogs (Figure 3); 4 of these had splenic hemangiosarcoma as well, whereas the spleen of the remaining dog was not available for analysis. Benign liver changes in 13 dogs included nodular hyperplasia, hepatitis, hemosiderosis, hepatocellular swelling, congestion, centrilobular necrosis, bile stasis, bile duct proliferation, lymphoplasmacytic infiltration, mesothelial cell hypertrophy, lipogranulomas, and pigment granulomas (Figure 4).

No adverse effects were detected following administration of contrast agent. Benign and malignant processes in the spleen were not distinguishable via CHUS. Qualitatively, peripheral parenchymal enhancement was evident in all splenic masses, followed by enhancement of clusters of small vessels extending into the center of the mass. Data from the undifferentiated sarcoma were excluded from the comparison between hematomas and hemangiosarcoma. Quantitatively, comparisons of interval to peak intensity (P = 0.87), peak MPI (P = 0.19), and area under the curve (P = 0.55) yielded no significant differences between hematomas and hemangiosarcoma (Table 1). Time-intensity curves for splenic hematomas and hemangiosarcomas were similar (Figure 5).

Qualitative evaluation of the contrast enhancement pattern of the liver according to published guidelines revealed that 10 of 18 livers contained no ultrasonographically detectable nodules. Results of histologic analy-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hemangiosarcoma</th>
<th>Hematoma</th>
<th>Sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval to peak intensity</td>
<td>71.0 (45.0–97.0)</td>
<td>76.0 (68.2–83.8)</td>
<td>74.0</td>
</tr>
<tr>
<td>Peak MPI</td>
<td>65.6 (55.0–76.2)</td>
<td>53.3 (39.3–67.3)</td>
<td>68.7</td>
</tr>
<tr>
<td>Area under the curve</td>
<td>4,360.1 (3,999.8–5,260.4)</td>
<td>3,770.1 (2,549–4,691.2)</td>
<td>5,017.5</td>
</tr>
</tbody>
</table>

Differences between mean values for hemangiosarcomas and hematomas were not significant.
sis of these liver biopsy specimens were consistent with those of a benign process. Three of 18 livers contained nodules (hyperechoic in 2 dogs and isoechoic in 1 dog) that were ultrasonographically detectable before contrast agent was administered and that appeared isoechoic to the liver parenchyma during CHUS. Again, results for liver biopsy specimens suggested the nodules were benign. The remaining 5 livers contained nodules (hypoechoic in 2 dogs, hyperechoic and hypoechoic in 2 dogs, and hyperechoic in 1 dog) that were detectable before contrast agent was administered and that remained hypoechoic to the liver parenchyma during CHUS. All of these livers had histologic evidence of metastatic hemangiosarcoma.

Analysis of the quantitative liver data revealed no significant ($P = 0.31$) difference in interval to peak intensity among the 3 histologic classifications (histologically normal liver, benign nodules, and hemangiosarcoma; Table 2). However, there was a significant ($P = 0.01$) difference in peak MPI. Specifically, peak intensity was significantly ($P = 0.01$) lower in metastatic nodules versus histologically normal liver, whereas peak intensity of benign nodules was not significantly different ($P = 0.23$) from that of histologically normal liver parenchyma. In addition, there was a significant ($P = 0.03$) difference between the liver-to-lesion intensity ratio for histologically normal liver to benign nodules, compared with the ratio for histologically normal liver to metastatic nodules. Median liver-to-lesion intensity ratio for histologically normal liver versus metastatic (hemangiosarcoma) nodules was 2.3 (range, 1.4 to 4.1). That for histologically normal liver versus benign nodules was 1.2 (range, 1.1 to 1.4).

Time-intensity curves for histologically normal liver parenchyma, metastatic hemangiosarcoma nodules, and benign liver nodules revealed that enhancement was less evident in metastatic nodules, compared with enhancement in normal parenchyma and benign nodules (Figure 6). Sensitivity and specificity of CHUS for detection of metastatic liver nodules were both 100%.

Of the 12 dogs with splenic hemangiosarcoma, hepatic hemangiosarcoma, or both, 1 was immediately euthanatized and 11 underwent exploratory laparotomy. The dog with the undifferentiated splenic sarcoma was also euthanatized without exploratory laparotomy. Five of the 11 dogs that underwent laparotomy died or were euthanatized within 24 hours after surgery for a variety of intraoperative (hemorrhage, refractory arrhythmias, or suspected widespread metastasis) and postoperative (severe dyspnea secondary to aspiration pneumonia, suspected intracranial hemorrhage, or respiratory arrest) reasons. Six dogs survived the perioperative period, with a median survival time of 10 weeks (range, 1.5 to 24 weeks).

All 7 dogs with splenic hematomas underwent exploratory laparotomy and survived the perioperative period. Of the 7 dogs with splenic hematomas, 1 was euthanatized because of an unrelated cause (gastric dilatation-volvulus) 12 weeks after surgery, 1 was lost to follow-up 22 weeks after surgery, and 5 survived throughout the study period, with a median elapsed time since exploratory laparotomy of 29.8 weeks (range, 22 to 60 weeks).

![Figure 5](image1.png)

Figure 5—Time-intensity curves representing results of CHUS evaluation of splenic hematomas (gray line) and hemangiosarcomas (black line) in 18 dogs with a splenic mass with or without hemoabdomen. Notice that the curves are similar in appearance.

![Figure 6](image2.png)

Figure 6—Time-intensity curves for histologically normal liver parenchyma (black line), metastatic hemangiosarcoma nodules (dotted line), and benign liver nodules (gray line) as determined histologically in liver specimens from 18 dogs with a splenic mass with or without hemoabdomen. Metastatic nodules had lower MPI than histologically normal parenchyma and benign nodules.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal liver (Mean (95% CI))</th>
<th>Benign nodule (Mean (95% CI))</th>
<th>Hemangiosarcoma (Mean (95% CI))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval to peak intensity (s)</td>
<td>73.1 (67.1–79.1)</td>
<td>79.3 (63.2–105.4)</td>
<td>81.6 (74.8–88.4)</td>
</tr>
<tr>
<td>Peak MPI</td>
<td>76.0 (64.9–87.1)*</td>
<td>61.8 (50.5–83.3)</td>
<td>40.0 (30–50)*</td>
</tr>
</tbody>
</table>

*Values indicated are significantly ($P < 0.05$) different from each other.

Table 2—Mean values (95% CIs) from quantitative analysis of results of hepatic CHUS in histologically normal liver, benign liver nodules, and hepatic hemangiosarcoma after injection of contrast agent in 18 dogs with a splenic mass.
Results of histopathologic evaluation of the splenic hematomas in the dogs in our study indicated areas of hemorrhage and necrosis, often interspersed with foci of lymphoid hyperplasia and islands of hematopoietic precursor cells (extramedullary hematopoiesis). Lymphoid follicular hyperplasia is considered a precursor lesion to the development of splenic hematomas because hyperplasia disrupts local blood flow, causing blood pooling, hypoxia, and necrosis.38 Hemangiosarcomas were described as proliferations of malignant neoplastic endothelial cells that commonly contained areas of hemorrhage and necrosis. Therefore, it is likely that the areas of perfusion that were evident surrounding and penetrating the splenic hematomas represented regions of nodular hyperplasia interspersed among anechoic foci of necrotic, hemorrhagic tissue in hematomas. This pattern was similar to the combination of clusters of neoplastic cells surrounded by hematoma formation that was detected in splenic hemangiosarcoma tissues. The undifferentiated sarcoma in the present study consisted of densely packed neoplastic mesenchymal cells with moderate amounts of cytoplasm, and there was hemorrhage in the surrounding tissues, resulting in an appearance via CHUS that was similar to that of the splenic hemangiosarcoma and hematomas.

The appearance of the metastatic hemangiosarcoma lesions in the liver as visualized via CHUS in the present study was similar to reported findings in the veterinary literature.1,12 Metastatic lesions detected via conventional ultrasonography remained hypoechoic, and previously poorly visible metastatic lesions became hypoechoic to the adjacent, brightly enhanced ultrasonographically normal liver parenchyma, resulting in a readily detected increase in conspicuity. The histologically benign nodules (typically associated with nodular hyperplasia) became less conspicuous (isoechogenic to the liver parenchyma) after injection of contrast agent. The quantitative findings further corroborated these results, supported by the significant differences in peak MPI between the 3 histologic classifications of liver tissue. Liver-to-lesion intensity ratios were calculated for the liver because these ratios are ultimately what an ultrasonographer evaluates when viewing the images. The quantitative results (ratios) supported the qualitative results: at peak liver enhancement, the relative intensity of ultrasonographically normal liver versus a metastatic nodule was significantly higher than the relative intensity of ultrasonographically normal liver versus a benign nodule.

In the study reported here, contrast-enhanced ultrasonography was a highly accurate method of characterizing nodule images as malignant or benign in dogs with hemangiosarcoma, with an accuracy of 100%. Visual inspection of the nodules at peak enhancement allowed distinction between benign nodules and hepatic hemangiosarcoma in all dogs. All dogs with hepatic hemangiosarcoma had liver nodules that remained hypoechoic after administration of contrast agent, compared with the appearance of the surrounding liver parenchyma, whereas benign liver nodules became isoechogenic to the surrounding parenchyma and were therefore less conspicuous after administration of contrast agent. Alternatively, measurement of the liver-to-lesion intensity ratio appeared to be the best quantitative measurement in a clinical setting because it was not dependent on contrast dose, blood pressure, and imaging equipment, all of which might influence measurement of peak intensity. On the basis of the results of our study, a cutoff value of > 1.4 for the liver-to-lesion intensity ratio would be appropriate to differentiate between benign and hemangiosarcoma nodules.

The small number of dogs in the present study was a limitation, and results should be verified in a larger scale study. Another limitation is that CHUS and histologic findings could not be compared for each nodule, although an attempt was made to describe the precise location of nodules detected via CHUS and to obtain a biopsy specimen of the same lesion whenever possible. Ultrasound-guided core biopsies of specific lesions were considered contraindicated because of the risk of creating more severe hemorrhage and destabilizing a dog prior to surgery. For this reason, we could only compare existence or lack of metastatic nodules for the whole liver. Data regarding the outcome of the dogs in the present study supported the histologic findings: at the conclusion of the study, all dogs with splenic hemangiosarcoma had died or had been euthanatized, whereas 5 of 7 dogs with splenic hematomas were still alive.

**References**