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Detection of Active Intra-Abdominal Bleeding from Malignant Tumors in Two Dogs Using Contrast-Enhanced Ultrasonography

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Abstract : Contrast-enhanced ultrasonography (CEUS) has been applied to evaluate parenchymal organs in human and veterinary medicine. However, to our knowledge, there is no report on the identification of active bleeding and the bleeding site in veterinary clinical patients. Herein, we describe the use of CEUS in two cases of abdominal bleeding caused by ruptured lesions with malignant abdominal tumors. One dog had a splenic hemangiosarcoma, which had metastasized to the liver; the other dog had hepatic cell carcinomas in the left hepatic lobe, which were lobectomized, and another nodule was identified in the right hepatic lobe. Immediately after the rupture of these oncogenic lesions was suspected, CEUS was performed to identify the bleeding sites. The active bleeding sites were confirmed by hyperechoic pooling signs in the arterial phase, and extravasation could be observed within the defects showing hypoechoic perfusions in the delayed phase of the CEUS. Microbubbles were also observed in the ascites; thus, CEUS could detect the presence of hemorrhage and accurately identify the bleeding site. Collectively, the study findings suggest the usefulness of CEUS in emergent situations as it enables rapid and noninvasive evaluation of bleeding points in case of active bleeding in dogs.

***Key words* :** active bleeding, bleeding site, tumor, CEUS, dog.

Introduction

Contrast-enhanced ultrasonography (CEUS) is a diagnostic method that involves analyzing the perfusion patterns and clearance times of contrast agent microbubbles, which are smaller than red blood cells, in the intravascular space.[1] CEUS is mainly used in veterinary medicine to evaluate parenchymal organs such as the liver and spleen (1). It has been used clinically to evaluate the kidneys, pancreas, lymph nodes and visualize portosystemic shunts in dogs (1). CEUS can detect small, ill-defined lesions which cannot be detected with B-mode ultrasonography (US), and can objectively evaluate the degree of tissue perfusion and detect tissue changes through time intensity curves (1). However, a few adverse reactions with CEUS have been reported, and it is a costly modality in veterinary medicine (1).

Nevertheless, in human medicine, it is well known that CEUS enables not only the evaluation of lesions in the parenchyma of organs but also the quick detection of a bleeding site and the application of rapid treatment in emergent traumatic or nontraumatic hemorrhaging (2). CEUS has been used to confirm the presence of bleeding before and after interventions such as tumor excision and biopsy (3). Using CEUS, hemorrhages can be detected by observing active extravasation of the contrast agent, which shows echogenicity similar to the related vessel and spread at the bleeding site a few seconds after the injection (2).

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In veterinary medicine, several cases in which CEUS was used to evaluate parenchymal organs, have been reported. However, to the best of the authors' knowledge, there have been no published reports that clinically employed CEUS to identify active bleeding and locate its source in active abdominal bleeding. Therefore, this report describes the use of CEUS for active peritoneal bleeding in two dogs with abdominal malignancies.

Case 1

A 13-year-old neutered female Pekingese presented with a splenic mass and underwent an emergency splenectomy due to a sudden rupture of the mass. The mass was confirmed as a hemangiosarcoma through histopathological examination. About a month after the emergency splenectomy, abdominal hemorrhage and anemia recurred, and transfusion was performed at the local veterinary clinic. Then, masses were identified in the liver on US, and they were considered to represent metastases from the splenic tumor. The patient was transferred to our hospital to confirm whether surgical resection of the hepatic masses would be possible.

On physical examination, the body temperature, heart rate, respiration rate, and blood pressure were normal, except for the pain response, which showed resistance to abdominal palpation. Computed tomography (CT) (Aquillion 64^{TM}, Toshiba, Tochigi, Japan) was carried out to reevaluate the possibility of surgical resection of the newly identified metastatic lesions in the overall liver.

CT examination was performed under general anesthesia in sternal recumbency with the following scan protocol: 2.0

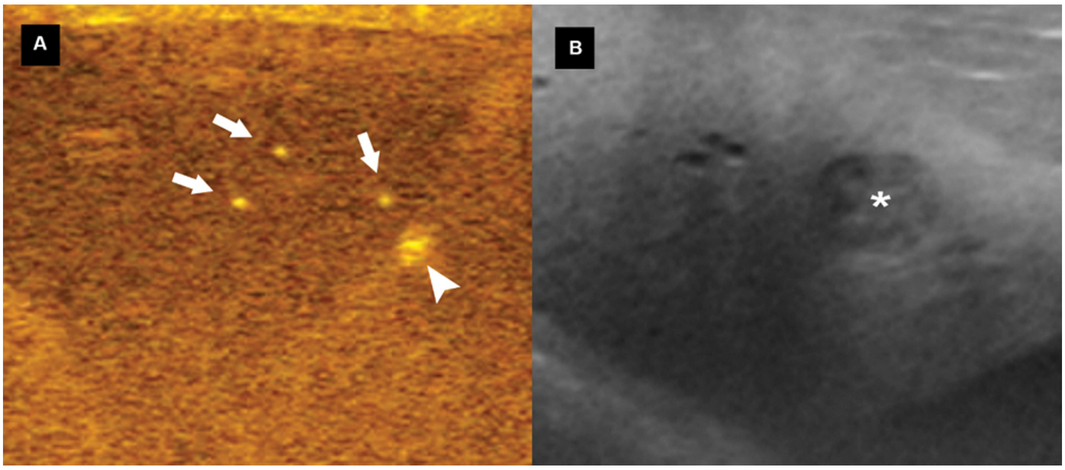

Fig 1. A, Contrast-enhanced ultrasonography of the lateral part of the right liver. Bubbles (arrow) are observed in the intraperitoneal echogenic fluid during the arterial phase, and extravasation (arrowhead) is identified at the border between the hepatic parenchyma and peritoneal fluid. B, Hepatic nodule in the left hepatic parenchyma. An irregular defect (asterisks) is continuously observed in the arterial and delayed phases.

mm slice thickness (thorax and abdomen), 120 kV, and 150 mA. Pre-contrast and triple-phase (arterial, 15 s; venous, 30 s; delayed, 120 s) scans were obtained after intravenous administration of iodinated contrast medium (Iopamidol; Iopamigita® 370 mg I/ml, Agfa HealthCare Imaging Agents GmbH, Germany) using a power injector. The hepatic nodules did not show any contrast enhancement with the iodinated contrast medium for all phases, but intense contrast enhancement was observed at several spots inside and at the periphery of the hepatic nodules. Considering the patient's history of hemangiosarcoma in the spleen, these findings were considered to indicate metastatic lesions. In addition, clot signs were identified around the adjacent nodules, which might have been due to hemorrhage resulting from rupture of the previous nodules, located in the right lateral lobe and caudate process at the hepatis level. However, there was no obvious evidence of intraperitoneal fluid.

Recovery from anesthesia and consciousness were normal after the CT exam, but sudden hypotension (70 mmHg, systolic), decreased vitality, and moderate anemia, with a packed cell volume (PCV) of 28% (reference range, 37.1-51.0%), were noted. A sudden hemorrhage resulting from the rupture of one of the hepatic mass was suspected, and we performed US (Aloka ProSound α 7®, Hitachi Aloka Medical Ltd., Japan) and CEUS to detect the hemoperitoneum and bleeding sites. US was performed using a 4.44-10 MHz microconvex and 4.4-13.3 MHz linear transducer. CEUS was performed using the same linear transducer. The mechanical index (MI) was set at 0.17-0.19 to minimize microbubble destruction.

In the two-dimensional US image, amorphous hypoechoic nodules were observed in the hepatic lobes without any blood flow signal in the doppler mode, and echogenic fluid was found in the abdominal cavity, particularly in the right cranioventral region. Subsequently, the nodules in the right and left liver lobes were examined using US with a contrast medium (Sonazoid (Perflubutane), Amersham Health, UK) (0.0125 mL/kg administered intravenously). Active bleeding due to mass rupture was confirmed, as bubbles were observed in the intraperitoneal echogenic fluid immediately after the intravenous injection (arterial phase) (Fig 1A). Extravasation in the lateral area of the right hepatic lobe was identified and was considered to be the bleeding source. Regarding the hepatic nodules were considered malignant, because irregular defects were continuously observed in the arterial (defined as 5-30 s after injection), delayed or parenchymal phases (defined as 10 min after injection) on CEUS (Fig 1B).

Notably, the 31% PCV in the ascites fluid obtained via paracentesis was higher than the previous PCV of 28% in the peripheral circulating blood, and a prompt blood transfusion was required. After application of a compression bandage and hemostatic treatment, the patient was transferred to a hospital where a matched blood transfusion was available; however, patient expired after a month because of worsened symptoms. Necropsy was not performed.

Case 2

A 10-year-old neutered female Welsh corgi presented with dyspnea, abdominal distension, and anemia. Seven months earlier, the patient was diagnosed with protein-losing enteropathy (PLE) with hypoalbuminemia. During steroid treatment for PLE, intraperitoneal hemorrhage occurred suddenly, and the patient underwent emergency surgery. During surgery, masses were identified in the left lateral and medial hepatic lobes. Under the emergency situation, left lateral and medial liver lobectomy was initially attempted, but the patient went into shock after resection of the lateral lobe, and we rapidly proceeded to close the abdomen after achieving hemostasis of the medial liver lobe. The resected hepatic masses were confirmed as hepatic cell carcinoma by histopathological examination.

After recovery, the patient underwent CT to further evaluate the remaining hepatic masses and potential metastasis. CT examination was performed under general anesthesia in sternal recumbency, similar to that in case 1. Hypoattenuated

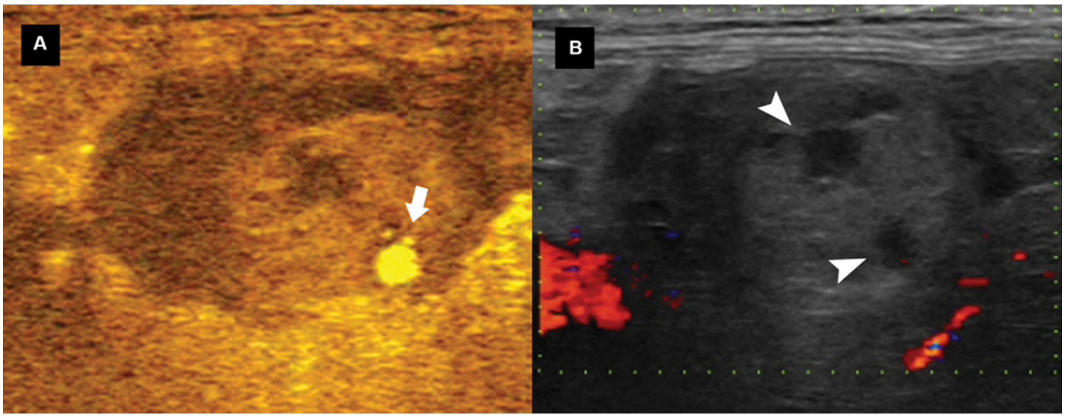

Fig 2. A, Contrast-enhanced ultrasonography of the hepatic nodule in the right hepatic lobe. There is a pooling sign (arrow) with intense contrast enhancement within the nodule. B, Several contrast defects (arrowhead) are noted within the hepatic nodule without the blood flow signal in the Doppler mode.

parenchymal lesions suspected of hematoma and necrosis were identified in the left medial lobe of the liver, some of which were connected to the quadrate lobe. Cysts or abscesses at the papillary process were additionally considered as diagnostic differentials. Regions of hypoattenuation in the caudate and left medial lobes were also seen, which indicated potential metastases. The hepatic lymph nodes were enlarged, and rim-enhancement was observed, which suggested malignancy. Identification, removal, and biopsy of the hepatic masses through exploratory laparotomy were recommended, but further diagnosis and operation were not conducted due to the owner's concerns regarding the surgery. Instead, the patient was monitored periodically.

About a month after the CT scan, as the patient showed sudden abdominal distension and lethargy, it was transferred to the emergency center to perform an evaluation for intraperitoneal bleeding. On immediate blood and imaging tests, the PCV was 36.8% (reference range, 37.1-51.0%), alanine transaminase level was 138 U/L (reference range, 5.8-83.3 U/L), and alkaline phosphatase level was 282 U/L (reference range, 0-97.9 U/L). These values were slightly higher than the normal ranges. In relation to the PLE history of the patient, the total protein level was 5.0 g/dL (reference range 5.7-7.5 g/dL), and the albumin was 2.2 g/dL (reference range, 2.6-4.4 g/dL), both of which were close to the lower limit of their normal ranges. A weak electrolyte imbalance was found, as the patient's sodium level was 138.9 mmol/L (reference range, 145.1-152.6 mmol/L) and the chloride level was 106.8 mmol/L (reference range, 113.2-122.9 mmol/L).

After the blood test, an abdominal US examination was performed, which showed a nodule that was not identified by previous imaging. This nodule was observed to be a heterogeneous echogenicity in the medial part of right lobe without blood flow signal and accompanied by a large amount of echogenic ascites was observed. A CEUS was also performed to assess intraperitoneal bleeding. Two injections of an ultrasound contrast medium (perflubutane) (0.0125 mL/kg administered intravenously) were administered to the nodule on the right hepatic lobe, which was identified on B-mode US; enhancement defects were noted in the arterial and delayed phases. In the arterial phase, pooling signs with very intense contrast enhancement within the nodule were observed (Fig 2A). In addition, contrast agent bubbles were observed in the ascites fluid due to extravasation, and active bleeding was detected at the hemorrhagic site in the nodule located in the right hepatic lobe. Contrast filling defects, without the blood flow signal in the doppler mode, were also identified within the nodule (Fig 2B).

The patient was indicated to undergo emergency surgery for hemostasis. However, the owner declined the surgery. The patient was subsequently monitored after the application of a compression bandage and hemostatic treatment. Although additional hemoperitoneum symptoms were not confirmed, we continued management by monitoring liver protein levels and clinical symptoms.

Discussion

Spontaneous bleeding due to liver disease can cause severe complications, but its clinical symptoms are not specific. Therefore, it is important to identify the cause and to treat the bleeding quickly. According to a study on humans with hepatocellular carcinoma, which accounts for the largest percentage of hepatic tumors in humans, hemorrhage due to tumor rupture occurs in 10% of fatal cases (4).

In our two cases of hepatic tumor rupture, we attempted to evaluate active bleeding using CEUS. In instances of active bleeding, hyperechoic pooling signs and extravasation could be observed within the defects showing hypoechoic perfusion at delayed phase on CEUS. The presence of microbubbles was also confirmed in the ascites fluid around the masses, thereby enabling determination of the presence of hemorrhage and accurate identification of the bleeding sites. According to a report of a human with hepatocellular carcinoma that was diagnosed using CEUS, extravasation in the lesion was not clearly recognized through CT, hence, the parenchymal bleeding site was not found (3). However, CEUS did reveal that the contrast agent had leaked from the lesion to the abdominal cavity (3). Moreover, hemostasis was achieved at the bleeding site through trans-arterial embolization without laparotomy, thus demonstrating the usefulness of CEUS in emergency situations (3). Although CT scan could provide considerable information for surgical treatment, with visualization of the entire abdominal cavity even in conditions such as subcutaneous emphysema, meteorism, and obesity (5). But by comparison, CEUS has merits of enabling a bedside diagnosis, particularly for patients with hemodynamic instability, and avoiding potential side effects such as hypersensitivity reactions to contrast agents, nephrotoxicity, and radiation exposure in CT scan (6). In addition, CEUS enables rapid diagnosis of active bleeding in emergencies and has the advantage of real-time imaging (7). Hence, given its low risk of side effects and real-time imaging capabilities, CEUS can be an effective modality to complement or replace CT.

Furthermore, CEUS can be performed not only in cases of tumor rupture but also in cases of acute hemorrhage caused by trauma. In a study of CEUS in dogs with active bleeding in the spleen parenchyma, varied patterns of dye extravasation were observed according to the type of perfusion method used (8). This study found that contrast enhancement in CEUS was reduced following contraction of the blood vessels and reduction in blood flow due to hemorrhagic shock.[8] In addition, according to a case of spontaneous rupture due to parasitic infection (nematode larvae) in a dog's kidney, the use of preoperative CEUS enabled the confirmation of hemoperitoneum and effective identification of the bleeding site (9). Thus, the detection of active bleeding and bleeding sites, regardless of the cause, are possible with the use of CEUS in veterinary medicine.

Several reports have been published on the application of CEUS in diagnosing malignant tumors in humans and animals (10-13). In our two cases, the characteristics of malignant tumors were observed during CEUS. Recently introduced ultrasound contrast agents have improved the quality of assessment of tumor vascularity compared with the low sensitivity of the existing B-mode US (10). In particular, perflubutane, a second-generation contrast agent provides not only real-time vascular imaging, but also a stable Kupffer phase based on the characteristic that microbubbles are phagocytosed by Kupffer cells (11). Thus, CEUS has an advantage in that it can be used to diagnose malignant tumors, especially in the liver, where Kupffer cells reside (11). Because hepatic malignancies generally do not involve Kupffer cells, defects of contrast in postvascular phase can be identified when compared with benign tumor (11). A study in human medicine revealed that the sensitivity and specificity of CEUS for diagnosing liver tumors and evaluating malignancy were higher than those of parenchymal dynamic CT (12). Herein, direct comparison with CT was impossible, but signs of contrast filling defect were identified in malignant lesions in the parenchymal phase, and observed on CEUS images. Moreover, the distribution of blood vessels and the presence of hemorrhage were evaluated based on the perfusion patterns. The findings described above may help establish a basis for the application of CEUS in the evaluation and treatment of hepatic tumors in dogs.

Conclusion

Our findings show that CEUS can be used not only to detect active bleeding in dogs but also identify the bleeding sites rapidly and precisely, thus enabling prompt administration of treatment. Furthermore, when intraperitoneal hemorrhage occurs due to tumor rupture, CEUS may permit timely evaluation of the parenchyma and characteristics of malignant of tumors.

Conflict of Interest

No conflicts of interest have been declared.

Abbreviations

CEUS (contrast-enhanced ultrasonography); CT (computed tomography); PLE (protein-losing enteropathy); US (ultrasonography); MI (Mechanical index).

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