Use of color flow Doppler ultrasonography to diagnose a bleeding neuroendocrine tumor in the gallbladder of a dog

Jitender Bhandal, BVSc; Laurie L. Head, DVM, DACVR; David A. Francis, DVM, MS, DACVS; Robert A. Foster, BVSc, PhD, DACVP; Allan Berrington, DVM, DACVP

Case Description—A 13-year-old neutered female Keeshond-cross was evaluated because of a history of melena, anemia, hematemesis, vomiting, and high serum liver enzyme activities over a 1.5-year period.

Clinical Findings—Abdominal ultrasonography revealed a hyperechoic mass in the gallbladder. In the gallbladder mass itself, a distinct linear blood flow pattern was detected by use of color flow Doppler ultrasonography.

Treatment and Outcome—A cholecystectomy was performed, and clinical signs resolved. Samples of the mass were examined histologically and immunohistochemically, and findings supported a diagnosis of neuroendocrine tumor of the gallbladder.

Clinical Relevance—Tumors of the biliary tree are a potential source of blood loss into the gastrointestinal tract. Color flow Doppler ultrasonography in conjunction with conventional grayscale ultrasonography may be useful in evaluation of the gallbladder in dogs. When echogenic material is detected in the gallbladder, it is important to evaluate the region for blood flow. (*J Am Vet Med Assoc* 2009;235:1326–1329)

A 13-year-old 20.6-kg (45.3-lb) neutered female Keeshond-cross was evaluated because of a history of anemia, hematemesis, vomiting, and high serum liver enzyme activities over a 1.5-year period. At the time of the initial evaluation, the dog was very weak, was reluctant to stand, and had hind limb muscle atrophy. Intravenous fluid therapy was initiated. A CBC, coagulation profile, blood gas analysis, thoracic radiography, and abdominal ultrasonography were performed. The results of serum biochemical analyses were supplied by the referring veterinarian. The dog had mild nonregenerative anemia (Hct, 0.303 L/L; reference range, 0.390 to 0.560 L/L) with a reticulocyte count < 1%. The coagulation profile results and platelet count were within reference limits. High serum activities of alkaline phosphatase (416 U/L; reference range, 4 to 113 U/L), alanine transaminase (802 U/L; reference range, 0 to 113 U/L), and γ-glutamyltransferase (38 U/L; reference range, 2 to 20 U/L) were detected; serum bilirubin concentration was also slightly high (8 µmol/L; reference range, 0 to 7 µmol/L).

Abdominal ultrasonography revealed that the liver was slightly hyperechoic and more coarse in appearance, compared with expected findings in a clinically normal dog. The gallbladder appeared distended, and the wall was 3.2 mm thick (thickness is typically ≤ 2 to 3 mm).[1] Within the lumen of the gallbladder, there was a circular hyperechoic mass that was attached by a sessile base to the ventral portion of the wall (**Figure 1**). By use of color flow Doppler ultrasonography, a linear blood flow pattern was identified within the mass. The bile duct was 13 mm in diameter proximally, and it narrowed to 6.1 mm at the duodenal papilla (bile duct diameter[2] is typically < 3 mm); irregular hyperechoic tissue extended from the gallbladder to the duodenum. The duodenum itself appeared unremarkable. Subjectively, the hepatic lymph node was large (12 mm thick); it was round rather than elongated (which is its typical appearance in clinically normal dogs), and the nodal parenchyma was mottled rather than homogeneous. Because the mass within the gallbladder was apparently solid tissue rather than gallbladder debris, the primary differential diagnosis was biliary carcinoma of the gallbladder with probable hemorrhage. The hyperechoic tissue in the bile duct was thought to represent hemorrhage and clot formation (**Figure 2**). This assumption was based on its solid hyperechoic appearance; no blood flow was detected in it by use of color flow Doppler ultrasonography. An exploratory surgery was recommended.

On observation during surgery, the gallbladder appeared grossly normal; however, a firm structure was palpated within the lumen. The cystic duct and the common bile duct were grossly dilated. The patency of the common bile duct was verified via a duodenotomy and passage of a 5-F red rubber catheter through the duodenal papilla into the common bile duct. There were blood and clots within the duodenum that appeared to be originating from the common bile duct. The gallbladder was bluntly dissected free from the liver parenchyma, a Satinsky clamp was placed at the distal end of the cystic duct, and the gallbladder was removed. A ligature of 3-0 polydioxanone suture was placed around the remaining portion of the cystic duct. The right lateral and medial liver lobes appeared grossly normal, but the left lateral and medial liver lobes had

From the Canada West Veterinary Specialists and Critical Care Hospital, 1988 Kootenay St, Vancouver, BC V5M 4Y3, Canada. Dr. Bhandal's present address is Department of Surgery, Michigan Veterinary Specialists, 29080 Inkster Rd, Southfield, MI 48034.

Address correspondence to Dr. Bhandal (jpsbhandal@gmail.com).

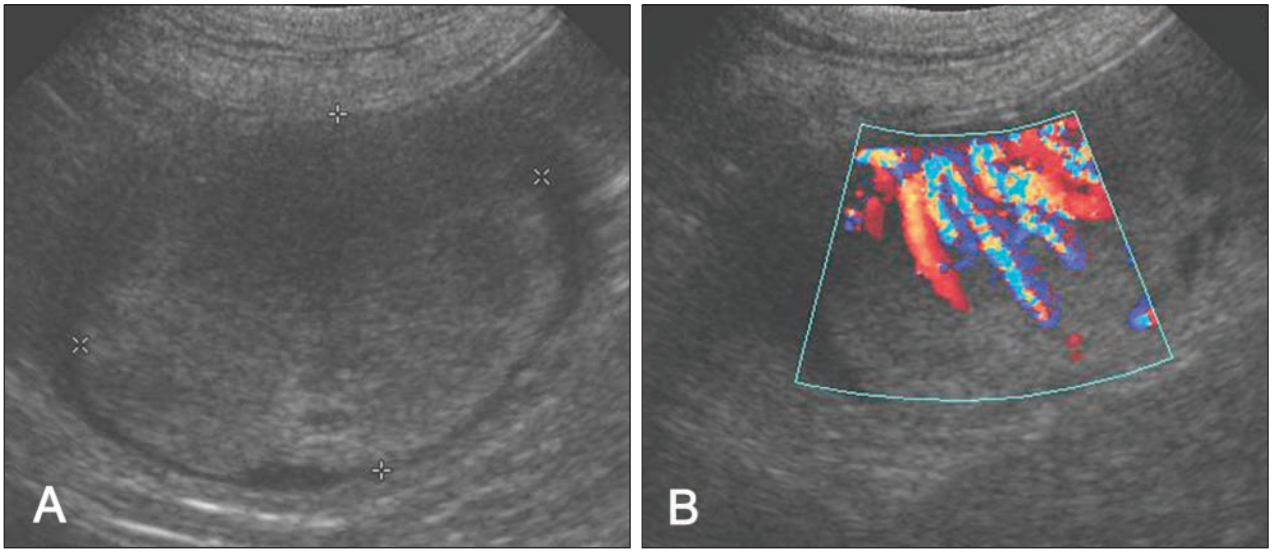

Figure 1—Ultrasonographic views of the gallbladder of a dog that was evaluated because of a history of melena, anemia, hematemesis, vomiting, and high serum liver enzyme activities over a 1.5-year period. A—Grayscale ultrasonographic image. Notice the circular hyperechoic mass within the lumen of the gallbladder. B—Color flow Doppler ultrasonographic image. A linear blood flow pattern is visible within the gallbladder mass.

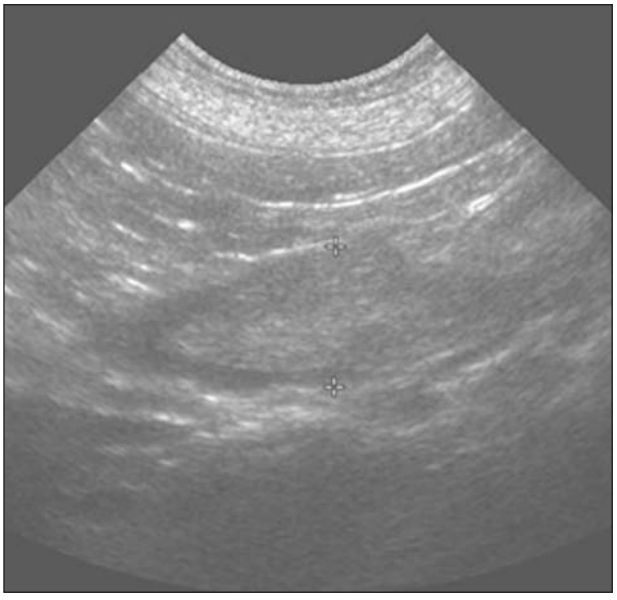

Figure 2—Ultrasonographic view of the bile duct in the dog in Figure 1. The bile duct is dilated and hyperechoic material is visible within the lumen. At surgery, this material was associated with hemorrhage from the gallbladder mass.

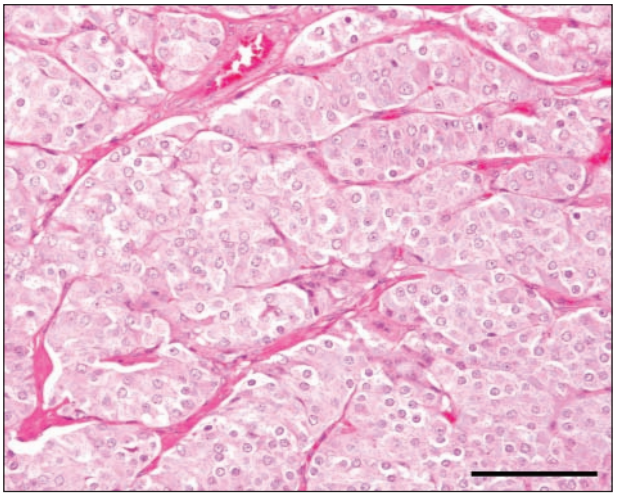

Figure 3—Photomicrograph of the gallbladder mass removed from the dog in Figure 1. Notice the cells of uniform size (with granular eosinophilic to amphophilic cytoplasm within well-defined cytoplasmic margins) that are subdivided into packets by a delicate fibrous stroma, typical of a neoplasm of neuroendocrine origin. H&E stain; bar = 100 μm.

a mottled white appearance. The large hepatic lymph node was isolated and removed. The gallbladder and lymph node as well as a biopsy specimen from the left medial liver lobe were submitted for histologic examination. The dog recovered from anesthesia and surgery uneventfully. Two days after surgery, the Hct was 0.30 L/L. The dog was discharged from the hospital on the third day after surgery; at this time, the dog's feces appeared normal. The dog was not returned for the recommended follow-up evaluation, but at an examination a year later, no abnormalities were detected via clinicopathologic analyses (Hct, 0.44 L/L). The owner reported no evidence of melena.

The mass in the gallbladder was approximately 1.5 cm in diameter; it originated from the wall of the gallbladder and projected into the lumen, where it was covered by a thin layer of biliary epithelium. It was well circumscribed and expansile in its growth behavior. It was composed of a diffuse sheet of evenly sized cells that were divided into packets by a fine fibrous stroma. Individual cells had well-defined cytoplasmic boundaries, an abundance of eosinophilic cytoplasm that was finely granular, and nuclei that were round and centrally located. The cells had a stippled chromatin pattern and occasional small nucleoli; the mitotic rate was < 1/10 hpf. Anisokaryosis was evident; many cells were typically 2 times as large as other cells, and some cells were 4 times as large (**Figure 3**).

Sections of the mass underwent immunohistochemical staining with antibodies against chromogranin, low–molecular-weight cytokeratin 7, neuron-specific enolase, and synaptophysin. The cells did not stain for cytokeratin 7, stained strongly for chromogranin, had partial and variable staining for neuron-specific enolase, and stained weakly for synaptophysin. These findings were consistent with a neuroendocrine tumor (carcinoid). There was a combination of inspissated mucous and blood within the lumen of the gallbladder. The wall of the gallbladder was approximately twice as thick as that expected in a clinically normal dog; mild epithelial hyperplasia was present with numerous lymphocytes and plasma cells beneath the epithelium. Occasional lymphoid follicles were detected. The medullary sinuses within the hepatic lymph node were markedly distended with edema, and some erythrophagocytosis was evident. Examination of sections of the liver biopsy specimen revealed evidence of congestion, necrosis, and focal scarring. The histologic findings in the lymph node tissue were consistent with lymphadenitis and edema with no evidence of metastatic disease.

Discussion

In humans and canids, neoplasia of the gallbladder or the extrahepatic biliary tract is very rare and neuroendocrine tumors are exceptionally rare.[3–7] Intrahepatic bile duct carcinomas can develop in cats and dogs.[8] To our knowledge, only 3 cases of gallbladder carcinoids or neuroendocrine tumors in dogs and cats have been reported in the veterinary medical literature.[9–11] Neuroendocrine cells are present throughout the gastrointestinal tract, the biliary system, pancreas, and lungs. Neuroendocrine cells produce many hormones, including serotonin, adrenocorticotropic hormone, chromogranin, cholecystokinin, and secretin.[5,10–12] Diagnosis of neuroendocrine tumors is based on histologic features and results of immunohistochemical staining.[10] In contrast to carcinomas of the gallbladder and extrahepatic bile ducts, primary neuroendocrine tumors of the gallbladder are considered slow growing and affected animals have a good prognosis if metastasis to the other organs has not occurred.[5,9–11] Metastatic potential of hepatic neuroendocrine tumors in dogs has been reported[13] to be high, particularly regarding metastasis to the peritoneal wall and adjoining lymph nodes. If no intraperitoneal or distant metastasis has occurred and the tumor is localized to the gallbladder, the prognosis for these patients is considered good.[14]

The etiology of gallbladder neoplasia in dogs is unknown. In humans, parasites (eg, trematodes), sclerosing cholangitis, or other sources of inflammation may induce biliary cancer.[6,15–17] In any species, tumors of the biliary tree are a potential source of blood loss into the gastrointestinal tract.

The dog of this report had a history of melena and anemia. To our knowledge, this is the first report of these clinical signs in association with a neuroendocrine tumor in the gallbladder of a dog. Dogs with neoplasia of the biliary tree most commonly have a combination of chronic weight loss, persistently high serum liver enzyme activities, and persistent vomiting. These are relatively nonspecific signs, and disorders of the gallbladder and extrahepatic biliary tract in dogs and cats can easily be confused with other intra-abdominal disorders.[8] In the 3 reports[9–11] of neuroendocrine tumors of the gallbladder in 2 dogs and 1 cat of which we are aware, 1 dog had hematemesis; the other dog and the cat had nonspecific signs including weight loss, high serum liver enzyme activities, and vomiting.

Ultrasonography is the main tool for diagnosis of gallbladder disease in small animal patients. Differential diagnoses for an intraluminal echogenic mass in the gallbladder include a gallbladder neoplasm (benign or malignant), inspissated bile, gallbladder mucocele, and cholecystoliths. Generally, cholecystoliths and notable volumes of inspissated bile or gallbladder debris are in a dependent location within the lumen and cause distal shadowing ultrasonographically. A gallbladder mucocele is generally characterized by hyperechoic nonshadowing material that is suspended within the lumen of the gallbladder; there are linear stellate projections that attach the material to the gallbladder wall. No blood flow is evident in association with inspissated bile, a gallbladder mucocele, or cholecystoliths. In the dog of this report, the gallbladder was filled with a solid predominantly hyperechoic mass that did not cause distal shadowing ultrasonographically. There was a distinct linear pattern of blood flow within the mass that could be differentiated from respiratory noise artifact (ie, normal respiratory motion detected ultrasonographically). This finding emphasizes the importance of evaluating for blood flow when echogenic material is detected within the gallbladder. Color flow Doppler ultrasonography should be used in all patients undergoing ultrasonographic examination of the gallbladder because findings may be helpful in differentiating gallbladder debris from solid tissue and determining early treatment or the need for surgical intervention.

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