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Case Submission Form

NEVPC CASE # 11

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SIGNALMENT: 10 year-old, female spayed, Scottish Terrier dog

HISTORY:

A 10-year-old, female spayed Scottish Terrier dog presented for evaluation of a cavitated liver mass. The patient had historically elevated ALP and ALT levels of 3 years duration, with more recent increases in enzyme activity.

Abdominal ultrasound revealed diffuse mottling throughout the liver and a large, approximately 7 x 7 x 4 cm, focal, cavitated liver mass in the left lateral liver lobe. Multiple renal cortical cysts and splenic nodules were also detected. Previous ultrasound examinations reported a normal appearing urinary bladder; there is a history of urinary bladder neoplasia among littermates.

Thoracic and abdominal CT imaging was performed, revealing a large mass in the left lateral liver lobe, with a second smaller mass within the caudate liver lobe, and multiple ill-defined small densities within the lungs. The patient was taken to surgery, where the masses on the left lateral and caudate liver lobes were removed by a partial liver lobectomy, and additional biopsies of the left and right medial lobes were also taken for histopathology. The patient recovered uneventfully and was discharged 2 days following the surgery.

GROSS FINDINGS:

At the time of surgery, an approximately 10 cm diameter mass was noted on the caudal edge of the left lateral liver lobe. A smaller, approximately 5 cm diameter mass was present on the distal end of the caudate liver lobe. No additional abnormalities were reported.

HISTOPATHOLOGIC/CYTOLOGIC FINDINGS:

Left lateral liver lobe: Effacing and replacing the hepatic parenchyma is a large, expansile, multilobular, unencapsulated, variably cystic mass composed of polygonal cells arranged in haphazard anastomosing cords up to 6 cells wide, within a moderate fibrovascular stroma (hepatocellular tumor). Neoplastic hepatocytes have distinct cell borders, with a moderate amount of granular, eosinophilic cytoplasm, and round to oval, central nuclei with finely stippled chromatin and a single prominent nucleolus. Neoplastic hepatocytes exhibit mild anisocytosis and anisokaryosis, with occasional binucleated cells. Mitoses are 1 in ten 400X high powered fields. Multifocally, neoplastic cells surround large cystic structures containing clear space, filled with free erythrocytes, hemosiderin-laden macrophages, vacuolated hepatocytes, fibrous connective tissue, and large tubular structures lined by a single layer of tall columnar epithelial cells with abundant, vacuolated, eosinophilic cytoplasm, and round, basal nuclei with finely stippled chromatin and a single prominent nucleolus (reactive biliary epithelium).

Multifocally associated with cystic areas are a second population of neoplastic epithelial cells arranged in islands and broad trabeculae, with variably distinct cell borders, a moderate amount of pale eosinophilic cytoplasm, and central, round to oval nuclei with finely stippled chromatin and occasional nucleoli. Cells exhibit mild anisocytosis and anisokaryosis. Mitoses are 1 in ten 400X high powered fields. In the parenchyma adjacent to the tumors, hepatocytes contain a moderate amount of intracytoplasmic,

irregular, clear vacuoles (glycogen-type vacuolar hepatopathy), and sharply demarcated, clear, lipid vacuoles, which peripheralize the nucleus (macrovesicular lipidosis). The bile ducts are dilated multifocally.

The following immunohistochemical stains are applied:

HEPPAR1 stain for hepatocytes: The examined section of mass is composed of two populations of neoplastic cells. The first population has poorly organized cords of hepatocytes, while the second population consists of multifocal cystic areas lined by lobules of polygonal neoplastic cells. There is a sharp demarcation in immunoreactivity, in which all of the neoplastic hepatocytes exhibit strong membranous and intracytoplasmic immunoreactivity (internal positive control), while the lobular population of neoplastic cells adjacent to the cystic structures are diffusely non-reactive.

Cytokeratin 19 (CK19) stain for epithelial cells, including biliary and urinary epithelium: Approximately 80% of lobules of cells adjacent to cystic areas exhibit strong membranous and intracytoplasmic immunoreactivity.

Cytokeratin 7 (CK7) stain for epithelial cells, including biliary and urinary epithelium: Approximately 10% of lobules of cells adjacent to cystic areas exhibit strong membranous and intracytoplasmic immunoreactivity.

Uroplakin III (UPIII) stain for urothelium: There is moderate background staining and brown pigment (hemosiderin and lipofuscin) within macrophages. No neoplastic cells exhibit immunoreactivity.

Cytokeratin 20 (CK20) stain for epithelial cells, including biliary and urinary epithelium: No neoplastic cells exhibit immunoreactivity.

Positive and negative controls are adequate for all examined sections

MORPHOLOGIC/ETIOLOGIC DIAGNOSIS:

Liver:

1. Hepatocellular carcinoma, trabecular type, with intratumoral metastatic carcinoma
2. Mild, multifocal, nondegenerative glycogen-type vacuolar hepatopathy
3. Mild, multifocal macrovesicular hepatic lipidosis

DISCUSSION:

Histologic evaluation reveals a hepatocellular carcinoma containing apparent intratumoral metastasis with adjacent mild hepatic lipidosis and diffuse non-degenerative glycogen-type vacuolar hepatopathy within the left lateral liver lobe. Additional examined sections revealed hepatocellular carcinoma within the caudate lobe, though no intratumoral metastases were noted these sections. Vacuolar hepatopathy (VH) is a common syndrome in dogs, usually reflecting hepatocellular cytosolic glycogen accumulation, observed histologically as swollen hepatocytes with clear and wispy cytoplasm, which stains positive with PAS.⁽¹⁾ VH is typically associated with iatrogenic administration or endogenous overproduction of glucocorticoids, though inflammatory, neoplastic, and infectious disorders may also cause VH.⁽²⁾

Scottish Terriers are known to develop progressive vacuolar hepatopathy with elevated alkaline phosphatase (ALP) activity.^(1,3) In a retrospective study, examination of 97 Scottish Terrier liver biopsies revealed that approximately 70% of biopsies had marked vacuolar hepatopathy.⁽⁴⁾ Multiple etiologies have been posited for this breed predisposition, including non-clinical hyperadrenocorticism,⁽⁵⁾ neoplasia, hepatobiliary disease, physiologic stress⁽²⁾, and genetic mutation.⁽⁴⁾

It has been noted that progressive VH leads to diffuse hepatic remodeling, as vacuolated hepatocytes undergo individual hepatocyte necrosis, with subsequent hepatic cord remodeling and formation of parenchymal nodules.⁽⁶⁾ Associations have also been made between VH, hepatic fibrosis, and hepatocellular carcinoma (HCC).⁽¹⁾ In a recent study, it was found that 39 of 114 (34%) of Scottish Terriers with vacuolar hepatopathy had HCC detected at surgery or necropsy, or via abdominal

ultrasonography.⁽⁶⁾ Histologic findings in this study suggested that HCC was seemingly preceded by dysplastic hepatocellular foci (which were noted in other examined sections of liver from this patient).⁽⁶⁾ The study also found increased sex hormone concentrations (progesterone and androstenedione), supporting adrenal gland hyperactivity in 88% of dogs tested.⁽⁶⁾ From these results, it was postulated that an inherited genetic disorder affects adrenal steroidogenesis, with subsequent ALP induction, hepatocellular glycogen accumulation, progressive VH, and ultimate formation of HCC.⁽⁶⁾

Hepatocellular carcinoma is the most common primary hepatobiliary neoplasm in the dog where it is often a solitary lesion but also may be multifocal and less commonly metastatic. Diagnosis is often initiated because of prominent increases in alkaline phosphatase (ALP) or asymmetric hepatomegaly, pursued with ultrasound imaging that discovers of an intrahepatic mass lesion. However, this neoplasm is also serendipitously recognized during abdominal ultrasound or exploratory laparotomy initiated for some other health concerns. Clinical discovery of metastatic HCC is relatively uncommon. Published rates of metastasis are discordant, ranging from less than 25% to 61%, when present metastatic spread typically involves regional lymph nodes, lung, and peritoneum.^(7, 8) HCC was reported with a frequency of 0.46% in a retrospective study of 12,245 canine necropsies.⁽⁸⁾ However, of the 446 cases of HCC in over 33 years at Cornell University Hospital for Animals, 5% of these cases were Scottish Terriers, representing a 10-fold relative risk for HCC development in this breed.⁽⁶⁾

This case is unique in that the hepatocellular carcinoma contains a distinct separate population of neoplastic cells. These cells display histologic characteristics consistent with a carcinoma (formation of islands and trabeculae) different than the surrounding HCC. Characterization via immunohistochemistry reveals that these neoplastic cells are CK19++ +/-CK7+/HEPPAR1-/CK20-/UPIII- and distinct from the immunoreactivity of HCC cells. Collision tumor or cholangiocellular transformation of neoplastic cells was considered. We considered the sharp demarcation between HEPPAR1 positive cells and CK19 positive cells more consistent with a metastatic event rather than a graduation transformation of neoplastic cells. Although the cells are immunoreactive for CK19, with weaker immunoreactivity for CK7, the overall pattern (lack of acini and tubule formation) is not typical for cholangiocellular carcinoma. We also considered hepatic carcinoids. However, the age of the animal, and the lack of associated histologic features, including formation of rosettes and ribbons, cells with granular cytoplasm, frequent mitoses, and abundant fibrosis decreased the likelihood of this differential.⁽⁹⁾

Considering the breed of the patient, and the history of bladder neoplasia in littermates, a metastatic transitional cell (urothelial) carcinoma (TCC) was considered. Knapp et al. determined that the risk of canine TCC is higher in Scottish Terriers, with a 19-fold increased risk compared with mixed breeds.⁽¹⁰⁾ Additional risk factors for TCC include female sex, history of spay or neuter, and obesity.⁽¹¹⁾ Studies have linked TCC in Scottish Terriers to environmental risks, such as exposure to lawn chemicals.⁽¹²⁾ Association with exposure to topical flea and tick pesticides was investigated as well; however, no increased risk was suggested in this study, though older products could not be evaluated due to the low sample size in this case.⁽¹³⁾ In a separate study, consumption of green leafy vegetable was found to decrease the risk of developing TCC in Scottish Terriers.⁽¹⁴⁾ Hepatic metastasis of TCC to the liver has been reported in 7% of dogs undergoing necropsy; however, this pattern of intratumoral metastasis is unusual.⁽¹¹⁾

The neoplastic cells were not immunoreactive for CK20 or UPIII; however, CK20 is restricted to superficial and occasional intermediate cells of the bladder urothelium, with only 46% of primary and metastatic tumors exhibiting positive immunoreactivity in one human study.⁽¹⁵⁾ Additionally, the lack of uroplakin III expression does not necessarily rule out a TCC. In a recent study of 99 canine proliferative urothelial lesions of the urinary bladder, it was noted that 42% of grade 3 urothelial carcinomas exhibited loss of UPIII expression.⁽¹⁶⁾ It was also reported that the majority (71%) of urothelial carcinomas exhibited a patchy CK7 immunostaining pattern, with a minority of cases losing CK7 expression. Transitional cell carcinomas have also been reported to exhibit CK19 expression, further supporting the suspicion for a TCC in this case.^(17, 18)

A recent study identified a somatic mutation in the BRAF gene in 85% of sequenced canine TCCs.⁽¹⁹⁾ BRAF mutations potently stimulate the mitogen activated protein kinase (MAPK) pathway, leading to

constitutive cell signaling, growth factor independent proliferation, and anti-apoptotic signaling.⁽¹⁹⁾ Using this information, analysis of urine for DNA containing the BRAF mutation has been developed, with a sensitivity of 85% reported.⁽²⁰⁾ Given the histologic findings, urine from this patient has been submitted for testing, and results are pending.

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