WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 20

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1060 William Moore Drive
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CASE I: T16-13553 (JPC 4084197).

Signalment: 10-year-old, female, spayed, American bulldog (*Canis familiaris*), canine.

History: Acute (sudden death). No additional history was available. A formalin-fixed tissue described as from a gallbladder mass was received.

Gross Pathology: Abdominal (gallbladder mass) was observed on gross examination.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): None provided.

Microscopic Description:
A formalin-fixed tissue from a gallbladder mass was received. A non-demarcated, non-capsulated neoplasm effaced the architecture of the gall bladder. The neoplasm is composed of closely packed sheets and nests of round cells supported on fine to ample fibrovascular stroma The neoplastic cells had indistinct cell borders and moderate, occasionally vacuolated, finely granular, eosinophilic cytoplasm. The nuclei were round, variably sized and had stippled
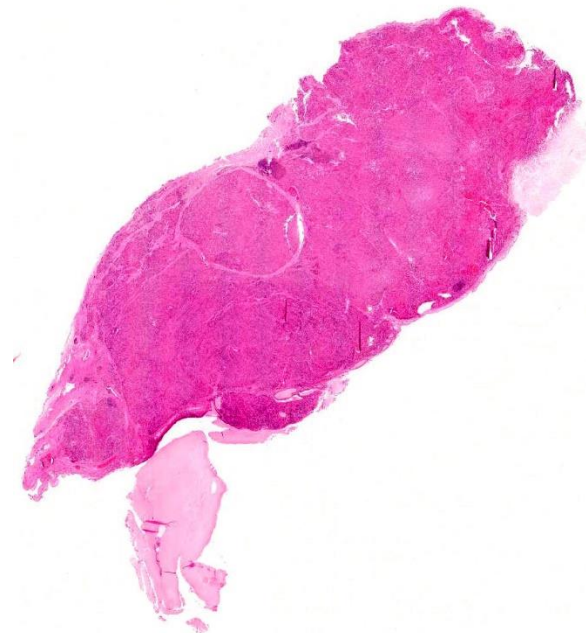
Gallbladder, dog. A large multilobular neoplasm has effaced the wall of the gallbladder (HE, 4X)

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nuclear chromatin. Occasional nuclei were hyperchromatic. Nucleoli were small to indistinct. Mitotic cells were very rare (<1 per 10x HPF). Anisocytosis and anisokaryosis were mild to moderate. Multifocally, aggregates of lymphocytes were observed in some sections. Dark staining cytoplasmic granules were demonstrated by special stains (Churukian-Schenk) for argyrophilic granules.

Contributor's Morphologic Diagnosis:
Neoplasm, neuroendocrine tumor (carcinoid), gallbladder

Contributor's Comment: Carcinoid tumors are uncommon neoplasms in humans and animals. The tumors arise from dispersed cells of the neuroendocrine system in the gastrointestinal tract, biliary system, pancreas and lungs. In dogs, hepatic, gastrointestinal and pulmonary carcinoids have been reported. Carcinoid tumors are reported in both sexes, several different breeds, and over an age range of 9-13 years. Extrahepatic biliary (gallbladder) carcinoid tumors are rare tumors in humans and very rare in dogs.[3,5,6,8] Carcinoid tumors in humans are potentially malignant and are usually slow growing tumors with a 5-year survival rate of 94% if localized, 64% if regional metastasis is present and 18% if distant metastasis has occurred during diagnosis.[7] Metastasis of carcinoids is also common in domestic animals and demonstrating the malignancy of the tumor. The tumor metastasizes in a similar manner to adenocarcinomas, through lymphatics and hematogenous routes. In dogs, small intestinal carcinoids frequently spread to the lung and pleura, liver, regional lymph nodes and pancreas.[3]

Since reports on canine primary gallbladder carcinoid are very rare, adequate information is unavailable to determine malignancies of
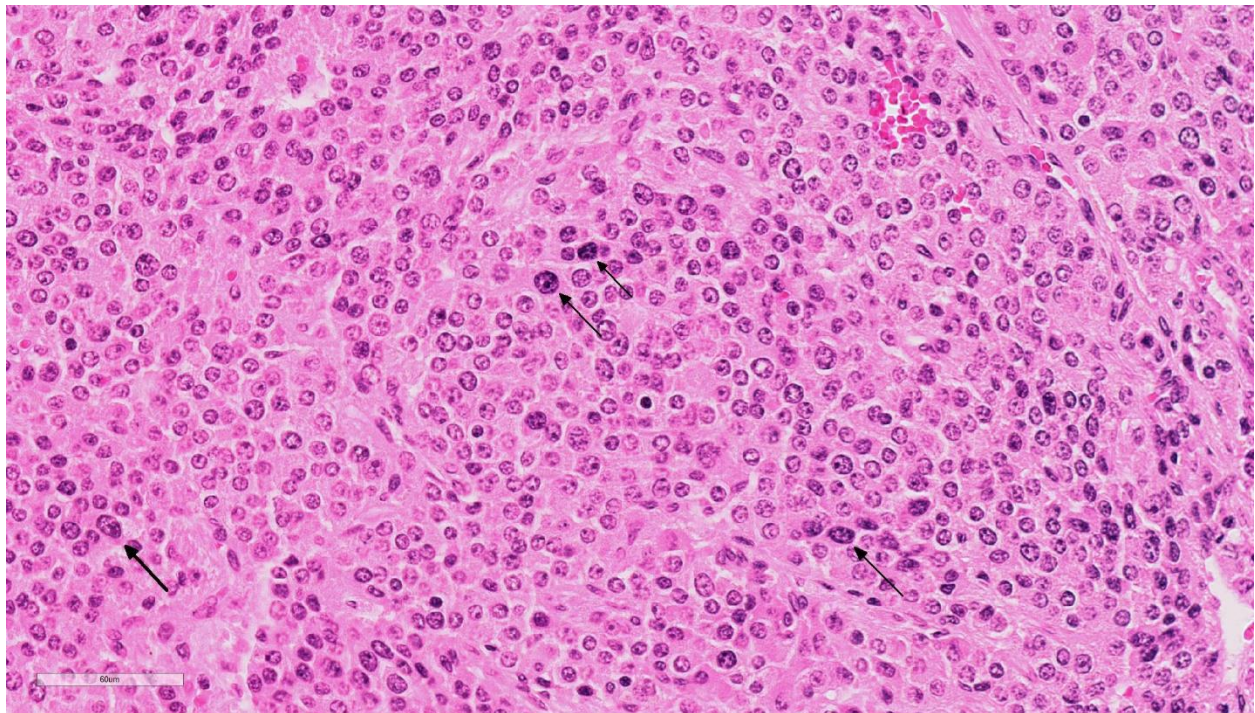
Gallbladder, dog. Neoplastic cells are polygonal and arranged in nests and packets. Karyomegalic cells are scattered throughout the section. Mitoses are rare. (HE, 400X)

primary gallbladder carcinoids in dogs. However, in one report, the presence of neoplastic cells within vessels of the gallbladder was suggested to indicate the potential of canine gallbladder carcinoid for metastatic dissemination.[8] Carcinoid tumors release various secretory products that often govern a clinical syndrome. For example, vasoactive amines released from the tumors may result in diarrhea, skin flushing, or cyanosis, hypertension, bronchoconstriction, pulmonary valvular stenosis, and right heart failure. Typical carcinoid syndrome as reported in humans has not been reported in domestic animals.[3]

Carcinoid tumors are commonly misdiagnosed especially if the tumor occurred in uncommon sites. Definitive diagnosis of carcinoid tumors requires immunohistochemistry, or special stains for argyrophilic granules or electron microscopy.[8] Immunohistochemistry against chromogranin A, synaptophysin and neuron specific enolase are often applied. The cytoplasmic granules stain positive with argyrophilic stains such as Churukian-Schenk and Grimelius stains. Therefore, special stains, such as Churukian-Schenk and Grimelius stains, which are readily available, are relatively inexpensive and should be applied in suspected cases of a carcinoid tumor.

**JPC Diagnosis:** Gallbladder: Carcinoid, American bulldog (*Canis familiaris*), canine.

Conference Comment: Carcinoids arise from neuroendocrine cells within mucosa of various organs, but most often the stomach and intestine. In animals, those neuroendocrine cells either secrete low-molecular-weight polypeptide hormones like secretin, somatostatin, and cholecystokinin or are part of a larger amine precursor uptake decarboxylation (APUD) group which

produces serotonin among other compounds. Although carcinoids are rare in animals, the most commonly reported locations in dogs are the duodenum, colon, and rectum. They are even more scarcely reported in other organs such as the lungs (more common in humans), liver, and gallbladder as in the present case.[2,11] As noted above by the contributor, there have been three recent reports of gallbladder carcinoids, all in dogs.[5,8] Historically, they have also been reported in the gallbladder of cats and cattle.

Within the liver, carcinoids arise from the neuroendocrine cell population associated with biliary epithelium and hepatic parenchyma; therefore tumors may form within the liver, extrahepatic bile ducts, or gallbladder.[2] Clinically, carcinoids most frequently cause secondary bowel obstruction and anemia from ulceration with subsequent hemorrhage. They can also be easily mistaken for polyps, especially when located at the anorectal junction. Diarrhea may be another associated finding, as a result of release of functional polypeptide hormones.[11]

Grossly, carcinoids are lobulated, firm, dark red to tan, and rarely larger than a few centimeters in diameter. They arise deep within the mucosa, commensurate with the location of neuroendocrine cells, causing submucosal nodules with ulceration and erosion of the overlying mucosa and often infiltrating transmurally into the mesentery.[11] In the liver, there is frequent intrahepatic spread with metastasis to local lymph nodes, peritoneum, and lung.[2]

Microscopically, carcinoids are composed of round to oval polygonal cells with abundant amounts of finely granular eosinophilic to finely vacuolated cytoplasm with round vesiculate nuclei and prominent nucleoli. In typical neuroendocrine fashion, the cells are
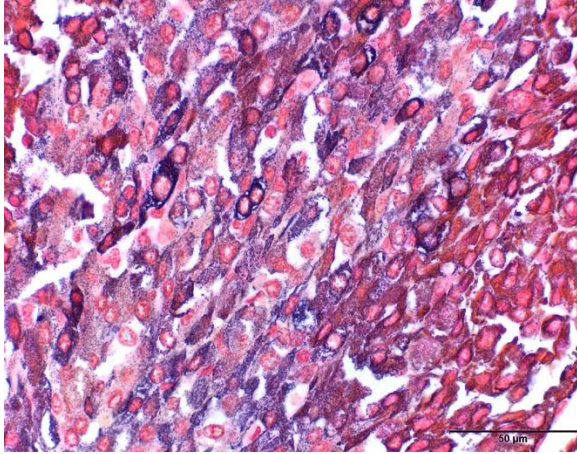
Gallbladder, dog. Neoplastic cells contain numerous dark argyrophilic granules (Churukian-Schenk, 400X) (Photo courtesy of: The University of Georgia, College of Veterinary Medicine, Department of Pathology, Tifton Veterinary Diagnostic and Investigational Laboratory, Tifton, GA 31793, USA
http://www.vet.uga.edu/dlab/tifton/index.php)

arranged in nests, and packets expanding the mucosa, submucosa, and muscularis; packets are surrounded by a fine fibrovascular stroma. Additional findings include: amyloid in between cells and adjacent to stromal blood vessels and megalocytes or multi-nucleated neoplastic cells. A potential histologic differential diagnosis is intestinal mast cell tumor, owing to the presence of numerous granules.

Ultrastructurally, carcinoid cells have numerous dense, round to oval, variably sized, membrane-bound intracytoplasmic secretory granules with abundant rough endoplasmic reticulum (RER) and a plasma membrane with interdigitating processes.[11] Mast cell tumors have less dense granules that can vary from homogenous to scroll-like admixed with large clear vacuoles (degranulation) and less RER.

The diagnosis of carcinoids is based on the following: neuroendocrine microscopic pattern, cytoplasmic granules which are argentaffinic (reduce silver solution to metallic silver after formalin fixation) and

argyrophilic (reduce silver solution to metallic silver after being exposed to a pre-reduction step)[10] granularity, immuno-histochemical identification of secretory products, and unique ultrastructural appearance. Histochemical and immuno-histochemical (IHC) reactivity can vary, especially if there is significant autolysis prior to fixation (common in the gastro-intestinal tract). However, in decent quality specimens, carcinoid cells are positive for neuron-specific enolase (NSE) and chromogranin as well as immunopositive for peptides being produced (e.g. serotonin). Those peptides may also be identified in circulation further supporting an endocrine diagnosis. Carcinoid cells are negative for periodic acid-Schiff and the granules do not exhibit metachromasia with Giemsa stains, further differentiating carcinoid from intestinal mast cell tumor.[11]

In humans, several paraneoplastic syndromes have been identified, such as: cutaneous flushing, diarrhea, bronchospasm, and systemic fibrosis. Fibrosis is concerning as it can lead to dysfunction of the pulmonary and tricuspid valves followed by regurgitation and right heart failure. This syndrome, termed "carcinoid syndrome", is most common in patients that develop carcinoids in their respiratory tract. The right side of the heart is affected because monoamine oxidases in the lungs fail to inactivate vasoactive substances, and these vasoactive substances lead to the aforementioned cutaneous flushing and bronchospasm which are key clinical indicators.[9] The mechanism of fibrosis in humans is unclear but is postulated to result from interactions between tumor cells and fibroblasts or hormones and peptides secreted by the carcinoid.[4]

Conference participants discussed the differences between benign and malignant carcinoids, which, according to the

moderator, are much more common in the liver (in his experience). In this case, with a history of a single mass and no evidence of vascular invasion in the submitted sections, this carcinoid is likely benign. Additionally, attendees reviewed the origin of carcinoid tumors and the moderator cited an article[1] which stated that proliferating cholangiocytes may take on a neuroendocrine phenotype, as well as initiate vessel proliferation, which may facilitate metastasis.[1] Shunt dogs, for example, tend to have proliferation of cholangiocytes and arterioles at the same time.

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http://www.vet.uga.edu/dlab/tifton/index.php

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CASE II: 20327-15 A or C (JPC 4099033).

Signalment: 2-year-old male neutered Cocker spaniel (*Canis familiaris*), canine.

History: In the 6-months prior to death, the dog had periodic bouts of inappetence, dark stools, and jaundice. The submitting veterinarian performed a necropsy and

Liver, dog: A rectangular section of liver is presented for examination. There is a vague nodularity even at low magnification. (HE, 5X)

submitted formalin-fixed liver to ALPC for examination.

Gross Pathology: Liver: Small and firm with numerous 1-2 mm, nodules throughout the surface.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):

ALT 168 (12-118 U/L)
ALP 172 (5-131 U/L)
Total Bilirubin 2.4 (0.1-0.3 mg/dL)
Albumin 1.6 (2.7-4.4 g/dL)
BUN 4 (6-31 mg/dL)
Urine bilirubin 3+
Bile acids pre-meal: 18.8 (<10.0 umol/L)
Bile aids post-meal: 21.8 (<20.0 umol/L)
Leptospirosis titers: Negative
Copper: 1460.0 ppm (dry weight)
Measurement was performed on formalin-fixed tissue.
COPPER DIAGNOSTIC LEVEL: Canine: Liver (DW) – normal 120-400 ppm; deficient < 80 ppm; toxic > 1,500 ppm.
**Several profiles were run throughout the 6-month duration of the illness. The most significant of these results are listed above.

Microscopic Description:

Liver: Diffusely, the normal hepatic architecture is replaced by numerous regenerative nodules that are up to 3.5 mm in diameter. Regenerative nodules are separated and surrounded by bridging tracts of fibrous connective tissue. Fibrous tracts contain a moderate proliferation of bile ducts and/or oval cells that are frequently mixed with individual or small clusters of hepatocytes. Small numbers of lymphocytes and plasma cells, few macrophages, and rare neutrophils are also multifocally scattered throughout fibrous tracts. Diffusely, there is marked loss of portal triads with few recognizable portal triads remaining within fibrous tracts. Bile canaliculi throughout the regenerative nodules are frequently expanded with bile. Multifocally, hepatocytes and Kupffer cells within nodules and fibrous tracts contain small to moderate amounts of yellow-gold to gold-brown pigment. Small to moderate numbers of hepatocytes within regenerative nodules and fibrous tracts exhibit macrovesicular vacuolation, characterized by single or small numbers of discrete, clear, intracytoplasmic vacuoles. Occasionally, portal veins and lymphatics are markedly dilated, and occasionally, sinusoids exhibit mild congestion.

HALLS BILE: Bile canaliculi, hepatocytes, and Kupffer cells within regenerative nodules and to a lesser extent fibrous tracts are often distended with bile pigment.
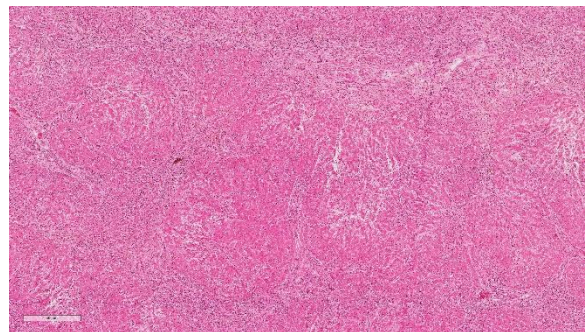
Liver, dog: At higher magnification, the parenchyma contains numerous regenerative nodules bounded by fibrous connective tissue. (HE, 70X)

RHODANINE: Frequently, hepatocytes and Kupffer cells within regenerative nodules and fibrous tracts contain small to moderate amounts of copper.

MASSON'S TRICHROME: Diffusely, delicate strands of collagen separate and surround individualized hepatocytes and hepatocyte clusters within fibrous tracts.

PRUSSIAN BLUE: Small numbers of Kupffer cells scattered throughout regenerative nodules and the fibrous tracts contain small to moderate amounts of iron pigment.

GOMORI'S RETICULIN: Diffusely, the normal lobular architecture is lost within both the regenerative nodules and the fibrous tracts. Reticulin fibers within the fibrous tracts are more numerous than the reticulin fibers within regenerative nodules and are also haphazardly arranged. Reticulin fibers within the fibrous tracts often separate and surround collagen bundles and dissect between or completely surround individual and small groups of hepatocytes and/or inflammatory cells.

Contributor's Morphologic Diagnosis:
Liver: Multifocal, widespread lobular dissecting hepatitis with marked fibrosis; mild, mixed inflammation; diffuse micronodular regeneration; moderate cholestasis; and occasional lymphatic ectasia

Contributor's Comment: The arrangement of the reticulin fibers within fibrous tracts indicates lobular dissection of the parenchyma. The lobular dissection and the proliferation of bile ducts within fibrous tracts is compatible with a diagnosis of lobular dissecting hepatitis, a form of cirrhosis most often seen in young adult dogs.[2,3,5,6] This form of chronic hepatitis has recently been reported in the American Cocker Spaniel.[3] Cocker Spaniels are at
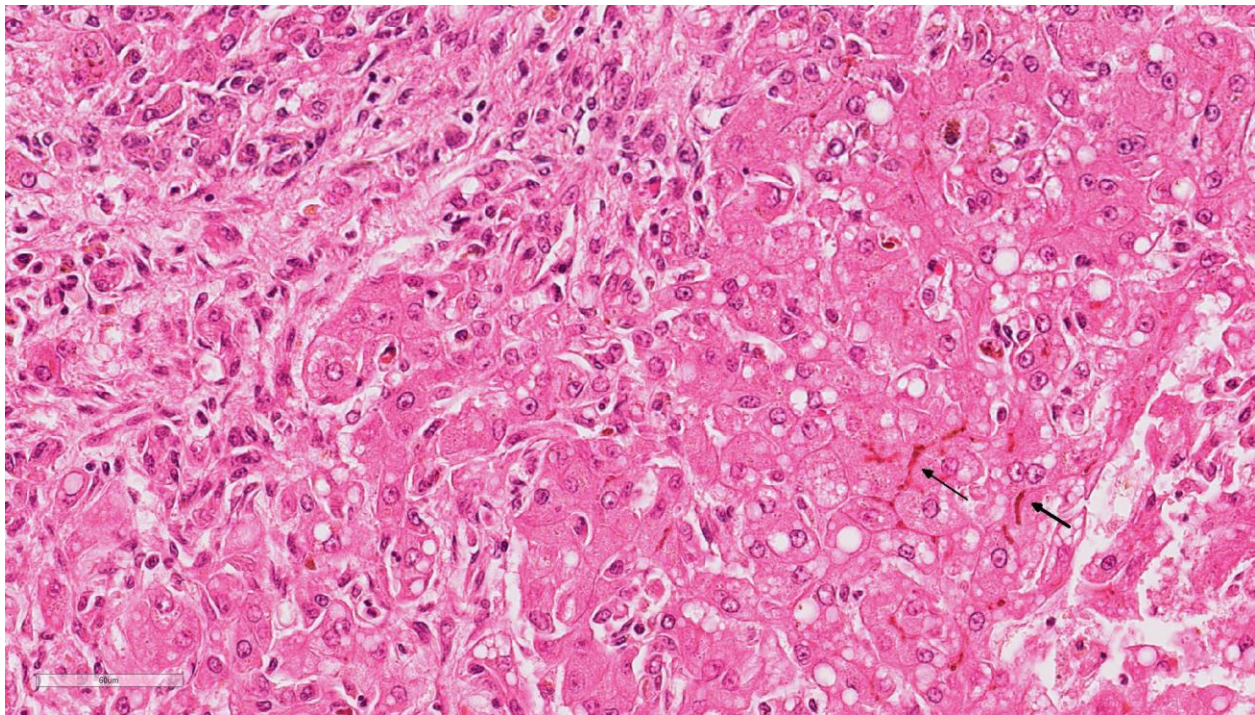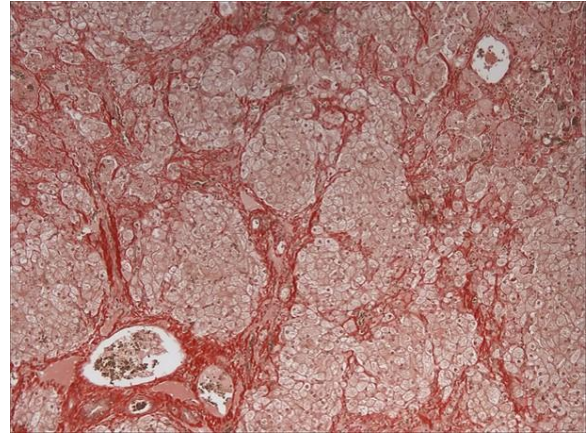

Liver, dog: High magnification demonstrates the extensive portal fibrosis and minimal inflammation at upper left, and adjacent dissection of small groups of hepatocytes. Small groups of dissected hepatocytes are often bordered directly by fibroblasts. There is cholestasis at the edge of the regenerative nodule (arrows). (HE, 400X)

increased risk for early onset chronic hepatitis that quickly becomes cirrhotic. Males are preferentially affected, and most of these dogs are diagnosed as young adults.[6] Disease is typically advanced at presentation, and most of these dogs lack signs of liver disease prior to the development of portal hypertension and ascites, the most common presenting sign.[3,6] Since disease is typically advanced at presentation, most dogs die within a few months of diagnosis.[6] In addition to cirrhosis, the condition is characterized by marked bile duct proliferation, a mild necroinflammatory response, and inconsistent copper retention.[2,3,5,6] Hepatic copper retention may occur as a primary injury or secondary to cholestasis. Since copper retention is inconsistent in this condition, most cases are attributed to cholestasis (which was prominent in this case).[2,6] The cause of chronic hepatitis in the Cocker Spaniel remains unknown. Because of the breed association, a genetic component has been postulated, and an α1-antitrypsin deficiency has been suggested, but remains unproven.[2,3,6]

JPC Diagnosis: Liver: Bridging fibrosis, diffuse, severe with marked micronodular hepatocellular regeneration, biliary hyperplasia, cholestasis, and diffuse hepatocellular lipidosis, Cocker spaniel (*Canis familiaris*), canine.

Conference Comment: Chronic hepatitis has frequently been reported in English and American Cocker Spaniels and English Springer Spaniels. Recently, American Cocker Spaniels have been reported to have lobular dissecting hepatitis, a distinct pattern of chronic hepatitis which has been most frequently identified in young dogs with ascites and acquired portosystemic shunts resulting from portal hypertension. Grossly, these two conditions have distinct

Liver, dog: A reticulin stain at low magnification demonstrates thick bands of reticulin fibers often following portal areas which divides the parenchyma into regenerative nodules. (Reticulin, 40X)

differences. Chronic hepatitis and cirrhosis appears grossly as small, firm livers with multiple regenerative nodules, whereas, in lobular dissecting hepatitis, the liver is also small and pale with fewer hyperplastic nodules.[1]

Microscopically, there is also variation in pattern between these two conditions. Chronic hepatitis is characterized by moderate to severe portal hepatitis with inflammatory infiltrates (lymphocytes, plasma cells and fewer neutrophils) and variable degrees of portal and bridging fibrosis. Lobular dissecting hepatitis, on the other hand, is characterized by dissection of hepatic parenchyma by reticulin and fine collagen fibers, subdividing individualized and small groups of hepatocytes. Fibroblasts, suspected of hepatic stellate origin, are prominent along sinusoids. Regenerative nodules may also be present, but not as consistently as with chronic hepatitis. In contrast with chronic hepatitis, portal inflammation and periportal fibrosis is not a prominent feature in lobular dissecting hepatitis.[1]

As mentioned by the contributor above, α1-antitrypsin deficiency has been suggested to

play a role in English Cocker Spaniels. α1-antitrypsin, a plasma glycoprotein, is a member of the serine proteinase inhibitor superfamily, and an inhibitor of neutrophil elastase. In humans, α1-antitrypsin deficiency leads to misfolded forms of α1-antitrypsin which accumulate in the rough endoplasmic reticulum of hepatocytes and form PAS-positive globules. The damage of hepatocytes resulting from accumulation of this protein has been associated with neonatal hepatitis, juvenile cirrhosis, and adult hepatocellular carcinoma in humans.[1]

A recent study of this condition identified the spindle cells producing reticulin and collagen fibers as myofibroblasts, as a result of their immunopositivity for anti-α-smooth muscle actin and anti-vimentin antibodies. Additionally, the reticular fibers produced by these cells were strongly positive for anti-collagen type III and type IV antibodies with positivity to anti-fibronectin and anti-laminin antibodies continuously along the basement membrane of the sinusoids of remaining hepatic cords and in between hepatocytes.
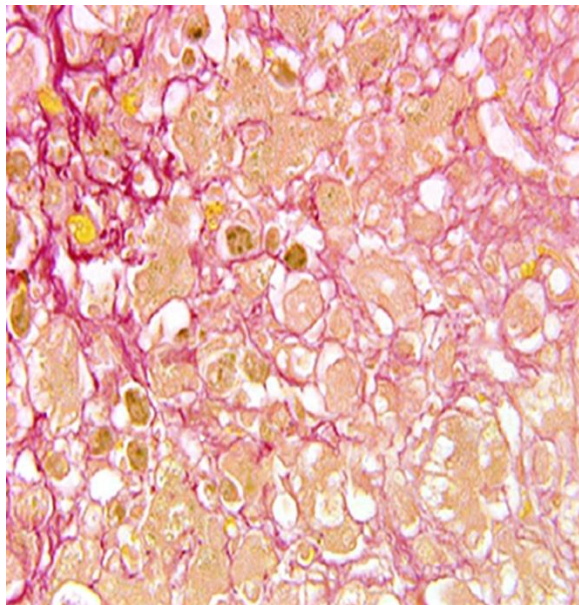
Liver, dog: A reticulin stain at high magnification demonstrates fibers often following surrounding individualized hepatocytes. (Reticulin, 400X)

This study also suggests that expression of fibronectin and laminin occurs before the deposition of reticulin between hepatocytes indicating an active extracellular matrix which contributes to the architectural damage.[4]

During the conference, the moderator reviewed patterns of cholestasis, observing that extrahepatic obstruction leads to circumferential fibrosis around bile ducts and biliary hyperplasia. The cholestasis in this case is likely due to fibrosis (intrahepatic obstruction) within the liver.

Conference attendees reviewed a case report[3] about American cocker spaniels with lobular dissecting hepatitis and noted that those dogs frequently have ascites that does not affect them as rapidly as other dogs with chronic hepatitis. Additionally, this report subdivides lobular dissecting hepatitis. According to this particular classification, the moderator believes that this particular casecase would fall in the subcategory of bridging fibrosis or micronodular cirrhosis. One unusual finding was the moderate amount of copper present in this case, whereas the dogs in the case study did not have any copper accumulation.

Contributing Institution:
http://www.aad.arkansas.gov/veterinary-diagnostic-lab

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CASE III: 10621-16 (JPC 4101304).

Signalment: 15-year-old male neutered Domestic shorthair (*Felis catus*), feline.

History: Necropsy organ samples were submitted to the Diagnostic Laboratory from a euthanized cat with a history of emaciation, jaundice, and difficulty breathing.

Gross Pathology: None provided.
Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): None provided.
Microscopic Description:
In the lung, many of the alveoli are filled with solid sheets of small cells containing scant amounts of cytoplasm with round to oval nuclei. In some areas, there is marked anisokaryosis. Approximately six mitotic


Liver, cat: At low magnification, portal tracts are accentuated by a cellular infiltrate. (HE, 9X)

figures can be seen in ten high powered fields. Along with these neoplastic cells, there are numerous and scattered aggregates of lymphocytes and plasma cells. Numerous airways are partially filled with plugs of desquamated cells mixed with mucus and inflammatory cells. Marked alveolar emphysema is also observed in these sections.

In the liver, periportal areas are markedly expanded due to the presence of large numbers of a mixed population of leukocytes. Limiting plates of lobules are disrupted by this inflammatory infiltrate. Lymphocytes and plasma cells extend into adjacent sinusoids from the periportal areas. Additionally, there is disseminated cholangiolar hyperplasia. Surrounding numerous cholangioles, there is a marked scirrhous response. Some of the larger cholangioles are markedly undulating due to the hyperplastic change and contain a few neutrophils and desquamated epithelial cells in their lumens. A few small aggregates of macrophages containing small cytoplasmic lipid vacuoles are present near the areas of portal inflammation.

In the pancreas, multiple foci of nodular hyperplasia can be seen. These nodules are separated from normal pancreatic parenchyma by slender fibrous septae. Modest disorganization of exocrine pancreas
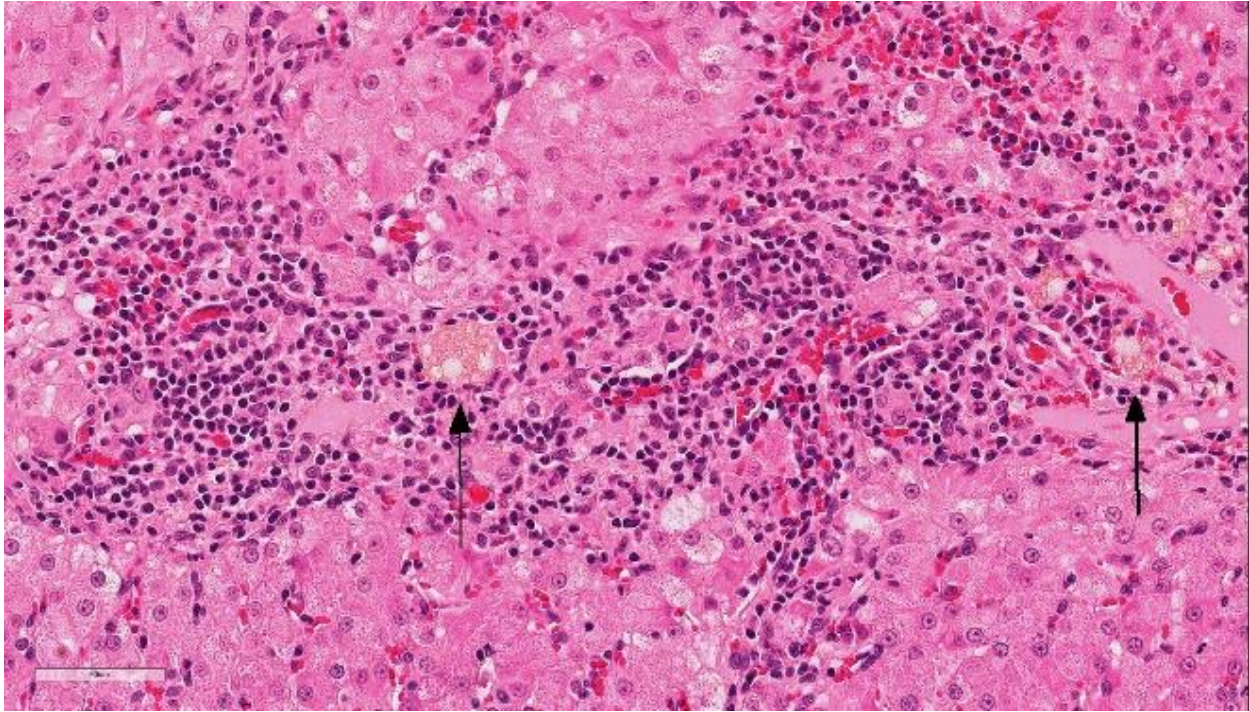
Liver, cat: Affected portal tracts display ductopenia as well as the formation of lipogranulomas. (HE, 315X)

is observed in these hyperplastic areas. There is moderate duct epithelial hyperplasia with small cystic formation in a major pancreatic duct. Occasional small lymphoplasmacytic aggregates can be seen in the interstitium of the pancreas and are sometimes associated with pancreatic islets.

Contributor's Morphologic Diagnosis:
1. Anaplastic small cell carcinoma – lung
2. Severe, chronic, lymphoplasmacytic, cholangitis
3. Marked multifocal nodular hyperplasia – pancreas
4. Mild multifocal lymphoplasmacytic pancreatitis

Contributor's Comment: While cholangitis is a relatively common set of liver diseases in cats[4,5] the overall prevalence is not well established as definitive diagnosis requires a biopsy and many cases are clinically unapparent during the early stages. In our case, the liver disease was identified coincidentally at necropsy as the cat was euthanized due to its lung cancer. This is a common finding as cholangitis does not typically result in mortality, but rather animals succumb to a concurrent disease process.[2]

Lymphocytic cholangitis in cats is a slowly progressive inflammatory lesion primarily of older animals that does not have a sex predisposition.[9] There have been a number of different pseudonyms used to describe the condition, although there is a proposal to standardize the classification scheme by the World Small Animal Veterinary Association (WSAVA) Liver Standardization group. Using their classification, the types of cholangitis can be subdivided into the following four groups: neutrophilic cholangitis, lymphocytic cholangitis, destructive cholangitis, and chronic cholangitis associated with liver fluke infestation.[8]

The most common type of cholangitis in cats is neutrophilic or suppurative cholangitis and the pathogenesis is through ascending bacterial biliary infections from the gastrointestinal tract.[5,8] These cases have portal neutrophilic infiltrates being the primary component during the acute infections and mixed infiltrates including lymphoplasmacytic infiltrates during the chronic stages. Secondary changes include biliary fibrosis and biliary hyperplasia, as well as a low risk of hepatic abscesses.[8]

Lymphocytic cholangitis is the second most common type of cholangitis, [8] although other studies have ranked it more prevalent than the neutrophilic variant.[4] An etiology has not been established for this condition, although it has been associated with inflammatory bowel disease and pancreatitis, suggesting that it may have an immune-mediated component. Clinically ,lymphocytic cholangitis tends to be slowly progressive without overt clinical signs during the initial development. Liver enzymes, although they may be elevated, are not directly correlated to the degree of inflammation.[2] Lesion severity is not uniform across all liver lobes. [2] Some cases can also histologically mimic hepatic lymphoma[8,9] although there are notable key differences which delineate these entities. These differences include bile duct targeting, ductopenia, peribiliary fibrosis, portal B-cell aggregates, and portal lipogranulomas, which are features that are associated with inflammatory rather than neoplastic infiltrates.[9]

Destructive cholangitis is typically reported in dogs rather than cats and is associated with drug reactions, biliary toxins, and some viral infections. Unlike neutrophils and lymphocytic cholangitis, this variant can cause severe cholestasis up to the point of bile obstruction.

Liver fluke infestation can cause chronic cholangitis is cats. These cases tend to have dilated bile ducts with fibrosis and papillary hyperplasia and pleocellular inflammation and can predispose to the eventual development of biliary carcinomas.[8]

JPC Diagnosis: 1. Liver: Cholangitis, lymphocytic, chronic, diffuse, severe, Domestic shorthair (*Felis catus*), feline.
2. Liver, bile duct: Cholangitis, neutrophilic, proliferative, focally extensive.

Conference Comment: Feline cholangitis is relatively common and presents in three different varieties: neutrophilic, lymphocytic, and chronic (due to liver flukes). A fourth variety, destructive cholangitis, is most common in dogs and briefly discussed by the contributor above. Microscopically, aside from the inflammatory cell population, they all have similar effects on the hepatic parenchyma: inflammatory infiltrates within portal regions and subsequent periportal to bridging fibrosis and bile duct or oval cell proliferation.

Clinically, these three have distinct presentations. Neutrophilic cholangitis is caused by bacterial cholecystitis, pancreatitis, and inflammatory bowel disease. These cats typically present with an acute history of lethargy, inappetence, pyrexia, and jaundice. Liver fluke infestations in cats is variable depending on the animal's environment, but they infrequently cause clinical disease and are usually incidental findings at necropsy.[1] The literature frequently associates chronic fluke infections with pancreatitis, however, a recent study conducted on cats on St. Kitts debunked that theory. They found that cats infected with *Platynosomum* sp. Rarely induces pancreatic damage in cats, and that any chronic pancreatitis present was subtle
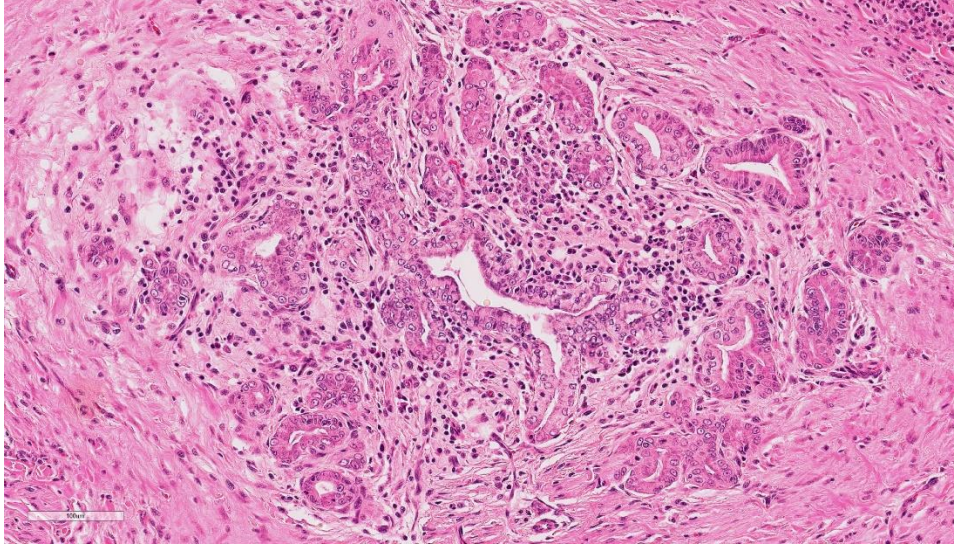
Liver, cat.   The section contains a central focus of proliferating bile ducts with irregular cuboidal to columnar biliary epithelium, mild lymphocytic inflammation, and marked circumferential fibrosis. (HE, 215X)

were clonal to oligoclonal as were their lymphoma cases. However, most (83%) lymphocytic cholangitis cases were polyclonal as expected.

Recent studies have sought to distinguish lymphocytic from neutrophilic cholangitis in cats with the least invasive modality. Unfortunately, ultrasonographic findings are identical for both conditions: diffuse liver and gallbladder hyper-echogenicity and enlarged pancreas.[7]

and most likely not related to the pathogenesis of platynosomosis.[6]

Lymphocytic cholangitis in cats is a relatively new condition characterized by lymphocytic infiltrates within portal regions and a biliary response with hyperplasia and duct destruction. The pathogenesis is unclear, but an immune-mediated disease has been proposed. One study identified T-cells as the predominant cell type with fewer B-cells admixed (at times forming secondary follicles). This study did not recognize eubacteria (using FISH) which rules out chronic bacterial cholangitis as the cause. Additionally, in cats, where T-cell hepatic lymphoma is common, this study found five microscopic features that distinguish lymphocytic cholangitis from lymphoma: (1) bile duct targeting, (2) peribiliary fibrosis, (3) portal B-cell aggregates, and (4) portal lipogranulomas.[9] Additional diagnostics include T-cell receptor (TCR) clonality assays.[3] However, this test may not be as reliable as previously supposed, Warren et al.[9] found unanticipated results in which a portion of cases with lymphocytic cholangitis

During the conference, the moderator identified circumferential fibrosis around bile ducts which are disproportionately small and lined by irregular biliary epithelium. In addition, some sections (not all) contained a single aggregate of irregularly shaped bile ducts with degenerating epithelial cells and neutrophils (resulting in the second conference morphologic diagnosis above). As one of the potential causes of lympho-cytic cholangitis is chronic neutrophilic cholangitis, the moderator speculated that this focus of atypical neutrophilic cholangitis might have been contributory to the overall condition. He further articulated that this focus does not appear neoplastic, and although there is a scirrhous response, the cells have normal organization and damaged biliary epithelium frequently produces that type of tissue response. Regarding the possibility of fluke infection in this case, the moderator stated that fluke lesions are large, often macroscopic, and result in marked

ductal fibrosis. Finally, he noted that it is important to rule out chronic neutrophilic cholangitis, a condition treatable with antibiotics, by culturing bile solids in these cases.

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**CASE IV:** 2012911922 (JPC 4032913).

Signalment: 11-year-old, female, spayed, Chihuahua (*Canis familiaris*), canine.

History: The dog was presented with a chief complaint of abdominal bloating. By abdominal ultrasonography, a large hepatic mass and hypoechoic lesion filling the peritoneal cavity were detected.

Gross Pathology: From surgical finding, the cystic mass originated from the hepatic left lateral lobe and filled the entire peritoneal cavity. The mass was the size of larger than 10 cm in diameters and was very soft and semitransparent milky to yellowish white mixed with blood. When made a cut in, a lot of mucus leaked, and the mass lost shape. The cut surface showed extensive myxomatous area. The site of liver attachment of the mass was harder and whiter than other area.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): None provided.

Microscopic Description:

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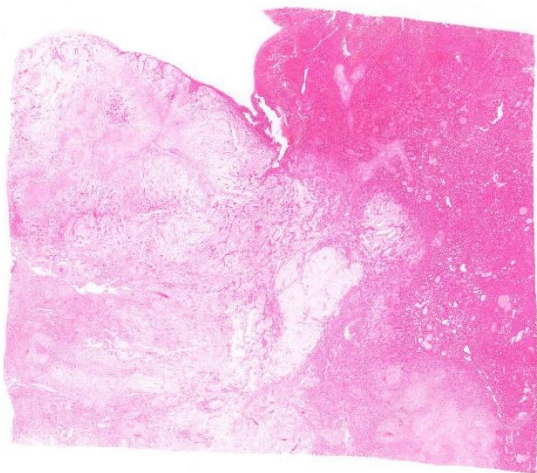
Liver, dog. Approximately 66% of the section of liver is effaced by a poorly cellular, markedly infiltrative neoplasm. (HE, 6X)

Spindle-shaped tumor cells chiefly proliferated in sheet or bundle with collagen fibers and mucinous matrix. Round or pleomorphic tumor cells also mingled with spindle-shaped tumor cells. The spindle-shaped tumor cells and collagen fiber were arranged in a concentric pattern in the perivascular areas. The tumor cells had round or oval nuclei of varying size with one or a few small to large distinct nucleoli. The cytoplasm of majority spindle shaped tumor cells was poor-marginated scant to abundant eosinophilic cytoplasm. Some pleomorphic to round tumor cells had several small or large vacuoles or a single giant vacuole like signet-ring-cell. Signet-ring shaped cells had usually thin cytoplasm compressed by a large vacuole and peripherally located nuclei. A few multinucleated tumor cells and large round cells were also observed. Mitotic figures were often seen. Hepatic cords and bile ducts were often remained between tumor cells within the mass, and the bile ducts were showed mild to moderate reactive hyperplasia. Mild hemorrhage and inflammatory cell infiltration were also seen. In the peripheral area of mass, thin spindle-shaped tumor cells proliferated with amounts of collagen fibers and invaded between hepatic cords.

On a Masson's trichrome stain, spindle-shaped tumor cells and collagen fibers were stained weak blue in contrast with dark blue at the edges of hepatic cords. However, vacuolated round tumor cells were unstained.

Immunohistochemically, spindle-shaped and vacuolated round tumor cells were strongly to moderately positive for vimentin. The spindle-shaped tumor cells were variably positive for alpha-smooth muscle actin. But all tumor cells were negative for desmin. Tumor cells of both types were intracellular strongly positive for laminin. The extracellular matrix was often weakly positive for vimentin, alpha-smooth muscle actin and laminin.

Contributor's Morphologic Diagnosis:
Liver: Ito cell tumor, malignant

Contributor's Comment: Mesenchymal tumors in liver are rare in human and animals. Hemangiosarcoma and leiomyosarcoma appear more commonly in animals.[7,12]

The normal liver tissue consists of four types of sinusoidal lining cells; endothelial, Kupffer, pit and hepatic stellate (Ito) cells.
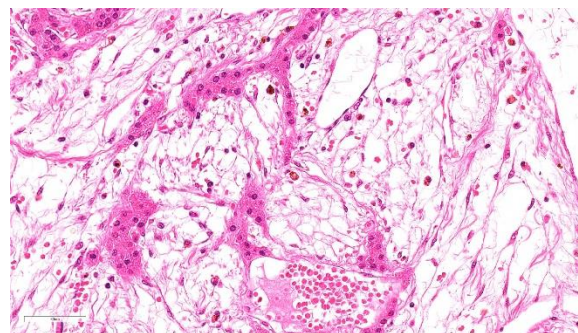

Liver, dog. The neoplasm is composed of widely-spaced spindle cells producing abundant mucinous ground substance. Neoplastic cells separated markedly atrophic cords of hepatocytes. (HE, 155X)

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Stellate (Ito) cell also called perisinusoidal cell, vitamin A-storing cells, fat-storing cells, lipocytes, and interstitial cells. It is difficult to distinguish between each cell types by the routine light microscopy, so immuno-histochemical stains and ultrastructural analysis are helpful.

Stellate (Ito) cells contain lipid droplets in cytoplasm, store vitamin A in lipid droplets, and play a role in the storage and regulation of vitamin A.[8] These cells have also another major role to produce extracellular matrix proteins.[11] During the proliferating process, stellate (Ito) cells lost fat droplets and vitamin A with myofibroblast-like appearance, and produce great amounts of extracellular matrix.[8,11] In immunohistochemical stains, stellate (Ito) cells without fat droplets and vitamin A are positive for desmin and actin/alpha-smooth muscle actin. It supports to identify proliferating stellate (Ito) cells as cells have myofibroblastic function.[4,8,11]

Present tumor was consisted of two principal histological types of tumor cells: spindle-shaped and vacuolated round. Those tumor cells proliferate within remaining hepatic cords and bile ducts and invaded hepatic parenchyma, producing collagen fibers. It seemed that this tumor arose from the hepatic sinusoid.
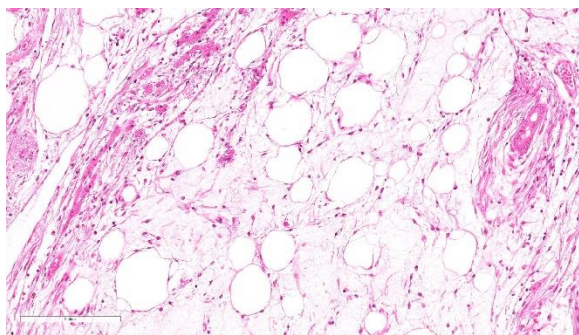
Liver, dog. Multifocally within the neoplasm, neoplastic cells are expanded by a large clear vacuole, resembling adipocytes. (HE, 350X)

Similar proliferative lesions have been reported rarely in animals[1,4,10,14,15] and humans.[13] These reports concluded or suggested stellate (Ito) cell origin from not only morphohistochemical features but also immunohistochemical and ultrastructural features.[1,4,10,15] Tumor cells in previous reports were immunoreactive for vimentin, desmin, α-smooth muscle actin, laminin,
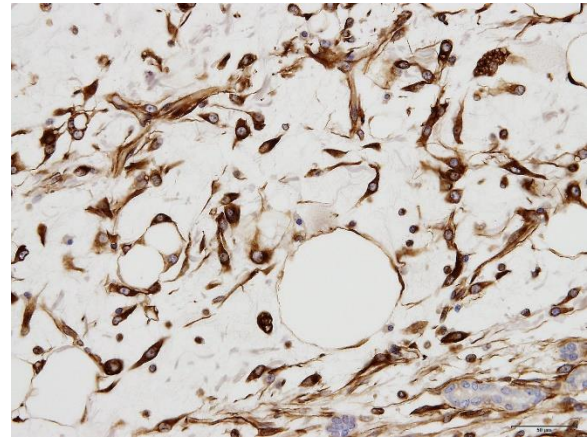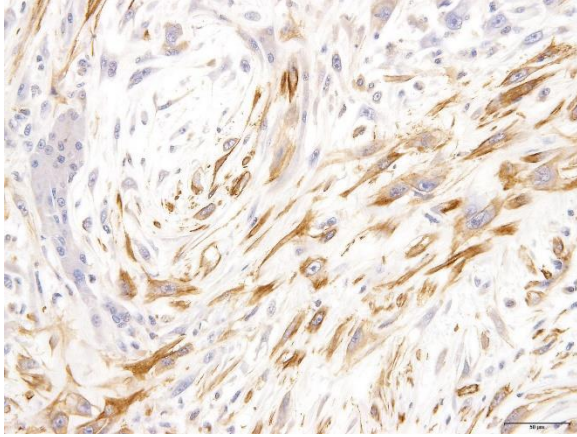

Liver, dog. Neoplastic cells exhibit strong cytoplasmic immunoreactivity for vimentin. Hepatocytes are negative. (anti-vimentin, 400X)

tenascin, and alpha B-crystallin. Tumor cells of present tumor show immunoreaction similar to previous reports with the exception desmin, suggesting that the present tumor originated from stellate (Ito) cells. Those previous reports suspected hepatic stellate (Ito) cell tumor was benign.[1,4,10,15] However, our present tumor had cellular atypia and many mitotic figures suggesting a malignant tumor.

Tumors suspected hepatic stellate (Ito) cell origin were diagnosed by use of various diagnostic term[1,4,10,13,15] because stellate cell had a lot of synonym. We diagnosed present tumor as malignant Ito cell tumor by references from the discussion of Stroebel P et al.[15]

Myxoid liposarcoma should be considered in the differential diagnosis.[17] Some tumor cells

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Liver, dog. Neoplastic cells exhibit moderately strong cytoplasmic immunoreactivity for smooth muscle actin. Unfortunately, the same stain run at the JPC was negative. (anti-smooth muscle actin, 350X)

of present case had small to large fat vacuoles in their cytoplasm. These tumor cells were suspended individually in a myxoid matrix similar to that seen in myxoid liposarcoma. However, the immunohistochemical profile including smooth muscle actin and laminin was different from our present case. Especially, laminin is a major compound of the hepatic extracellar matrix. In the repair processes of focal hepatic injury, laminin positive interstitial matrix are increased with Ito cells proliferation after infiltration of various inflammation cells.[12] Intra- and extra- cellular laminin expression were seen in the previous Ito cell origin tumors.[10,16] In addition, hepatic cords often remained in the tumor tissues. It seems that the tumor arise between hepatic cords and proliferated with separation of them. The histomorphologic and immunohistochemical characteristics supported that present tumor originated from Ito cells.

JPC Diagnosis: Liver: Sarcoma, poorly differentiated, with myxoid differentiation, Chihuahua (*Canis familiaris*), canine.

Conference Comment: Hepatic stellate (Ito) cells are remarkably diverse mesenchymal cells found between hepatocytes in the space of Disse and serve primarily as storage cells for lipid and vitamin A in large round vacuoles. The functionality of stellate cells does not end there, they also play a role in the following arenas: hepatic fibrosis, cytokine release, blood flow, and antigen presentation.[5]

When the liver is damaged, stellate cells become activated and take on a myofibroblastic phenotype, evidenced by the presence of immunohistochemically measurable α-smooth muscle actin, with concomitant loss of lipid vacuoles. In addition to the production of collagen and other extracellular matrix components (proteoglycans, fibronectin, and hyaluronin) activated stellate cells also release cytokines which are proinflammatory, profibrogenic, and promitotic.[5]

Stellate cells can affect sinusoidal blood flow by producing matrix and narrowing sinoisoidal lumina and by constriction of myofibroblastic stellate cells around endothelial cells. This act of constriction is induced by increased production of endothelin-1 by sinusoidal endothelial cells, which, in health, is balanced by vasodilation stimulated by nitric oxide also released by sinusoidal endothelial cells.[5]
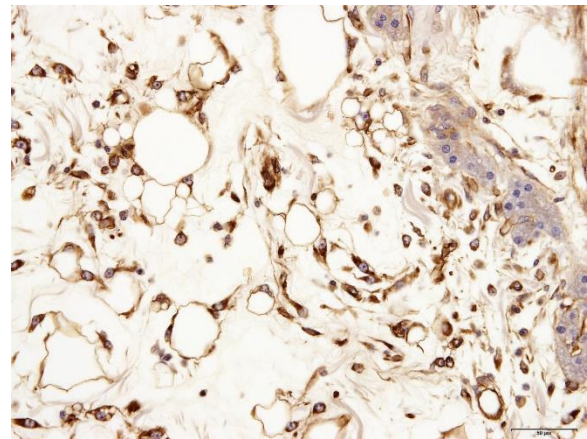

Liver, dog. Neoplastic cells exhibit strong cytoplasmic immunoreactivity for laminin. (anti-laminin, 400X)

Finally, stellate cells act as antigen-presenting cells in the liver much the same as Kupffer cells. In this realm, they mostly function to present lipid antigens to CD1-restricted T lymphocytes (like natural killer T-cells), but also present antigenic peptides to CD4+ and CD8+ T-cells with priming of CD8+ T-cells.[18]

Stellate (Ito) cell tumors are more common in mice than other domestic animals but are still rare.[3] Gross lesions are described as moderately firm, pale white nodules found within the liver parenchyma. Microscopically the nodules are composed of nodular aggregates of round to spindle cells that have dark oval nuclei and variably sized clear cytoplasmic vacuoles on a myxomatous matrix. These vacuoles are positive for oil red O and Sudan black staining, identifying them as lipid. Tumor cells are positive for vimentin, actin, desmin, and proliferating cell nuclear antigen. Additionally, the extra-cellular matrix stains positive for laminin and tenascin.[16] A key differential in rodents is Ito cell hyperplasia which is described associated with *Helicobacter* sp. infection and numerous other circumstances. The main difference is with hyperplasia there is usually concurrent Kupffer cell hyperplasia as well.[3]

The conference participants as well as the moderate (an internationally renowned hepatic specialist) had difficulty precisely diagnosing the neoplasm as well as identifying it as of Ito cell origin. The moderator first cited the gross findings which state that mucus leaked out of the mass when it was incised; in his experience, stellate cell tumors rarely have that much myxomatous material present. To further underscore the difficulty in attributing the origin to that of Ito cells, the moderator cited several articles. The first one discussed morphologic characterization of myofibroblastic cells in the liver – the portal myofibroblasts and stellate cells.[9] They found that desmin was
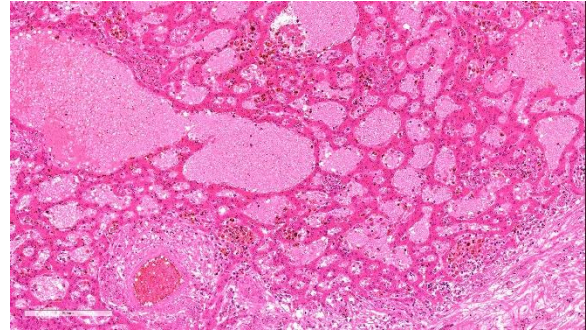
Liver, dog. There is marked sinusoidal dilation for at the periphery of the neoplasm as a result of markedly aberrant vascular flow and massive chronic congestion. (HE, 107X)

unreliable in differentiating between the two, but that stellate cells were generally negative for vimentin. This fact is in stark contrast with the present case, in which the neoplastic cells are vimentin-positive. Further, the moderator elaborated on the presence of abundant mucin grossly which he stated may be more representative of a hemangio-pericytoma, which like many types of mesenchymal neoplasma may demostrate a myxoid variant that is desmin negative (similar to this case).[2] Finally, mesenchymal hamartomas may have a similar appearance to this neoplasm with a loose stroma surrounding ducts. Mesenchymal hama-rtomas are developmental anomalies in the biliary system of horses and have also been reported in humans. Considering all facts, the moderator would prefer sarcoma (myxoid variant) rather than being specific about cell type.

In an effort to be as specific as possible, a battery of histochemical and immuno-histochemical stains were run including: Alcian blue, S-100, GFAP, smooth muscle actin, and desmin. The myxomatous matrix within the neoplasm is Alcian blue-positive but all other stains are negative. including smooth muscle actin, but which was positive for the contributor. In our stain, there is good internal control with strong intracytoplasmic

immunoreactivity of the smooth muscle cells in vessels and myoepithelial stellate cells within portal regions, but the neoplastic cells are diffusely negative. Furthermore, we consulted M.D. gastrointestinal pathology subspecialists who admittedly had not seen an Ito cell tumor and could find no report of such a neoplasm in the human literature. In their experience, this is consistent with a myxoid fibrosarcoma, but had poorly differentiated liposarcoma as a differential due to the presence of fat.

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