CYTOLOGY OF THE LIVER
Maxey L. Wellman, DVM, PhD, DACVP (Clinical Pathology)
Professor, Department of Veterinary Biosciences, College of Veterinary Medicine,
The Ohio State University, Columbus, OH, USA

SUMMARY

Fine needle aspiration (FNA) of the liver may be helpful in the diagnostic work-up and development of a treatment plan for small animal patients with suspected liver disease. As sensitive imaging procedures have become more widely available, the use of cytology for evaluation of abdominal organs has increased. Guidance of needle placement by imaging can enhance the probability of collecting a diagnostic sample, especially from smaller masses or those that are difficult to palpate. Although sometimes diagnostic, it is important to remember that cytology is a screening tool that complements other clinical information. Only a small sample of tissue is evaluated, so it is difficult to assess the extent of involvement. Important architectural arrangements also cannot be assessed by cytology. Discordance between cytology and other clinical findings should be pursued by histopathology or other diagnostic techniques.

Indications for FNA of the liver for cytologic evaluation include generalized hepatomegaly or lobar enlargement, presence of a mass or nodular lesions, abnormal echogenicity, suspicion of neoplasia or inflammation, or confirmation of hepatic lipidosis or steroid hepatopathy. Potential complications include hemorrhage and seeding of neoplastic cells along the needle tracts, but these are rare. Platelet counts of < 20-50 x 10^9/L are viewed by some as a contraindication for a FNA of the liver. Although screening patients for hemostatic abnormalities may minimize the risk of hemorrhage, abnormal test results do not predict excessive bleeding in all patients, and hemorrhage can occur even if screening results are normal.

Normal Liver

Hepatocytes exfoliate readily and are distributed as single cells or in clusters. Normal hepatocytes are large (25-30 μ), polyhedral cells with abundant amphophilic cytoplasm that appears finely granular. Bile pigment and lipofuscin pigment granules can be present in hepatocytes that otherwise appear normal. Bile pigment appears as dark bluish-black to greenish-blue granules of variable size. It can be difficult to distinguish diffusely distributed bile pigment from the small bluish-green to greenish-black granules due to lipofuscin accumulation in lysosomes, which may be prominent in hepatocytes from older animals. Nuclei are round and centrally located, and have coarsely stippled chromatin and single, prominent nucleoli. Occasional binucleated cells may be present. Rare, rectangular crystalline intranuclear inclusions are of no known pathologic significance.

There may be occasional small clusters of cuboidal or columnar biliary epithelial cells, which have small amounts of light blue cytoplasm; round, basally located nuclei with condensed chromatin and inconspicuous nucleoli; and a relatively high nuclear to cytoplasmic ratio. Occasional hepatic stellate cells (perisinusoidal cells or Ito cells) are identified by the presence of
multiple lipid droplets. These cells store vitamin A, function as antigen-presenting cells, and likely are involved in fibrosis in cirrhosis. Occasional macrophages (e.g. Kupfer cells), small lymphocytes, and mast cells also may be present. Variably-size sheets of mesothelial cells can occur, and typically are characterized by their angular cell shape and an eosinophilic cytoplasmic border, although these sometimes may be difficult to differentiate from bile duct epithelial cells.

**Lipid and Nonlipid Vacuolation**

Lipid vacuolation most commonly occurs in cats with hepatic lipidosis. The clear, distinct vacuoles are from accumulation of triglycerides. The presence of lipid can be confirmed by staining with oil red O, but this usually is not necessary, especially if there are clinical findings and laboratory changes that support a diagnosis of hepatic lipidosis. Lipid vacuolation also can occur with metabolic disorders such as diabetes mellitus. The amount of lipid vacuolation may be mild, moderate, or marked. In severe hepatic lipidosis, it may be difficult to recognize the cells as hepatocytes. Congenital lipid storage diseases in young animals can cause diffuse hepatomegaly. FNA are characterized by widespread vacuolar change similar to the lipid that accumulates in hepatic lipidosis.

Nonlipid vacuolation, sometimes referred to as rarefaction (less density of the cytoplasm than normal), can occur from increased water as a result of ischemia, inflammation, cholestasis or infection, or from. Vacuolar change due to increased water content is called hydropic degeneration. Increased endogenous corticosteroids causing nonlipid vacuolation occurs most commonly in dogs with hyperadrenocorticism (Cushing’s disease); it occurs less commonly in cats. The cytoplasm has a lacy, wispy appearance, often associated with mild, moderate, to marked cell swelling. The vacuolar change may be mild, moderate, or severe, and does not always correlate with the severity of clinical signs or laboratory changes such as increased alkaline phosphatase activity often associated with increased corticosteroids.

**Nodular Hyperplasia**

Nodular hyperplasia occurs relatively commonly in older dogs and is characterized cytologically by slightly increased cell and nuclear size, increased nuclear-to-cytoplasmic (N:C) ratio, increased variation in cell size (anisocytosis) and nuclear size (anisokaryosis), increased numbers of binucleated cells, and increased cytoplasmic basophilia. The hepatocytes may exhibit vacuolar change that resembles lipid or water or glycogen accumulation. There may be focal accumulations of macrophages containing lipid or lipofuscin; variable numbers of neutrophils, lymphocytes, and macrophages; and evidence of extramedullary hematopoiesis. It is difficult to differentiate nodular hyperplasia from other diseases that cause similar hepatic pathology, or from hepatocellular adenoma or well-differentiated carcinoma based on the cytology. The increased numbers of neutrophils and lymphocytes can be misinterpreted as inflammation. Histopathology is recommended for further evaluation of these types of lesions.

**Inflammation**

Neutrophils can be from blood contamination, inflammation, or both. A hemogram is helpful in determining if blood contamination is the only cause of increased numbers of
neutrophils. Neutrophils associated with inflammation sometimes are located in close association with clusters of hepatocytes, whereas those from blood often are more evenly distributed. Early stages of cholangiohepatitis, hepatitis, and hepatic abscesses are characterized by neutrophilic inflammation. Aspiration of a hepatic abscess can potentially induce peritonitis and should be carefully considered, especially if there is a focal hypoechoic lesion in a febrile animal. Bacterial organisms occasionally are present in liver aspirates.

Small lymphocytes predominate in lymphocytic and lymphoplasmacytic hepatitis and cholangiohepatitis, chronic lymphoid leukemia, and small cell lymphoma. It is difficult to distinguish inflammatory lymphocytic infiltrates from neoplastic proliferations of lymphocytes based on cell morphology if the lymphocytes are small. However, most cats with lymphocytic and lymphoplasmacytic hepatitis and cholangiohepatitis have normal peripheral lymphocyte counts, whereas most dogs and cats with chronic lymphoid leukemia have lymphocytosis. The lymphocyte count in dogs and cats with lymphoma involving the liver is variable. Immunophenotyping by flow cytometry or biopsy may be helpful for further evaluation.

Some viral, mycotic, and protozoal infections; drug and toxic injuries; and immune-mediated disorders are characterized by a mixed inflammatory infiltrate that includes neutrophils, macrophages, lymphocytes, plasma cells, eosinophils, and multinucleated giant cells. For example, feline infectious peritonitis causes mixed inflammation involving neutrophils, macrophages, and lymphocytes in variable amounts that can involve the liver. Liver aspirates from some cats with chronic cholangiohepatitis have a mixed population of neutrophils and small lymphocytes. Liver fluke infestation and eosinophilic enteritis may be characterized by eosinophilic inflammation. Canine infectious hepatitis can be associated with large, magenta intranuclear viral inclusions. Membrane-bound cytoplasmic invaginations sometimes occur with chronic liver disease and may appear similar to a nuclear inclusion.

**Other findings**

Cholestasis is characterized by the presence of linear casts of bile pigment, which appears greenish black. Bile casts often occur along the edges of hepatocytes and are especially visible within clusters of hepatocytes. These animals often have increased serum bilirubin. Hemorrhage and hemolysis sometimes are associated with increased hemosiderin, which appears as golden brown to bluish-black coarse granules of variable size. Hemorrhage and hemolysis typically are associated with other clinical and laboratory findings that support increased loss or lysis of red blood cells.

Necrosis can be associated with a diffuse blush-gray background, poor differentiation of nuclear detail, poor distinction between nuclei and cytoplasm, and karyorrhectic nuclei. Extramedullary hematopoiesis most commonly occur in the spleen, but sometimes is present in the liver, in which case there are variable numbers of myeloid and erythroid precursors, and occasional megakaryocytes.

Hepatomegaly can be associated with amyloidosis, which occurs when there is increased deposition of amyloid A protein produced by hepatocytes in response to chronic inflammation, or as a familial disease in some breeds of dogs and cats. Amyloid appears as amorphous
eosinophilic material between hepatocytes. The presence of amyloid can be confirmed with Congo red staining and detection of birefringence when the specimen is examination using polarized light.

In copper-associated hepatopathy, there may be visible greenish, somewhat refractile granules in hepatocytes. Copper usually appears lighter and more angular and refractile than bile pigment or lipofuscin. Confirmation by rubeanic acid on cytology, rhodamine staining of a liver biopsy, and copper determination in hepatic tissue is recommended for a definitive diagnosis.

**Neoplasia**

Primary epithelial tumors involving the liver include hepatocellular adenomas (hepatoma) and carcinomas and bile duct (cholangiocellular) adenomas and carcinomas. Hepatomas typically are associated with a large single mass involving one liver lobe. Cells from a hepatoma resemble normal hepatocytes on FNA, although there may be a mild increase in anisocytosis and anisokaryosis. It is very difficult to distinguish hepatoma from a regenerative nodule or even a well-differentiated hepatocellular carcinoma based on cytology, and this distinction sometimes is difficult histologically. Hepatocellular carcinomas also can be a single mass involving one liver lobe. The cells can appear relatively well-differentiated or very anaplastic. Features of anaplasia include moderate to marked variation in N:C ratio, cell size, and nuclear size. There may be increased numbers of binucleated and multinucleated cells. Vascular invasion or intrahepatic metastasis can be helpful in the histologic diagnosis of hepatocellular carcinoma.

Neoplasms arising from bile duct epithelial cells also can be benign or malignant, and the cytologic appearance of both can resemble normal biliary epithelial cells. The nuclear to cytoplasmic ratio usually is higher and less variable in these tumors than in hepatocellular neoplasms. Histologic confirmation is required for a definitive diagnosis. Histologic evaluation often is required for a definitive diagnosis.

Other neoplasms include neuroendocrine tumors, malignant tumors of mesenchymal tissue origin, hemolymphatic neoplasms, and metastatic carcinomas. Neuroendocrine tumors are rare. FNA may contain only nuclei from broken cells. Intact cells are large and have round nuclei with fine chromatin. Numerous fine eosinophilic granules sometimes are visible, and can be detected more easily with special stains. There may be moderate to marked variation in cell size, nuclear size, and N:C ratio. Confirmation with histopathology also may require special stains.

Lymphoma is the most common hematopoietic neoplasm involving the liver, usually as part of multicentric lymphoma. Neoplastic lymphocytes most often are large, round, individual cells with moderate amounts of basophilic cytoplasm. Nuclei are round to slightly irregular and have fine chromatin and prominent nucleoli. Immunophenotyping sometimes is used to determine if the cells are B or T lymphocytes. Infiltration of the liver with acute lymphoid leukemia can have similar appearing cells, but these animals usually also have non-regenerative anemia, neutropenia, and/or thrombocytopenia. Immunophenotyping also can be helpful in differentiating between lymphoma and acute lymphoid leukemia. The lymphocytes in chronic lymphoid leukemia involving the liver usually are small and often appear well-differentiated. As
mentioned above, it may be difficult to differentiate non-neoplastic lymphoproliferative diseases from chronic lymphoid leukemia or lymphoma involving a small cell type. A CBC, immunophenotyping by flow cytometry, and biopsy may be helpful in this distinction.

Mast cell neoplasia can involve the liver. The cytologic appearance of mast cells usually is very helpful in the diagnosis. However, normal liver contains mast cells, and these sometimes increase with inflammatory diseases involving hepatic tissue. If there is more than 1 mast cell per 100 hepatocytes, or there are aggregates of mast cells, the index of suspicion for mast cell neoplasia increases. Histiocytic sarcoma involving the liver is characterized by large, round to irregularly shaped cells with moderate to marked variation in cell size, nuclear size, and nuclear to cytoplasmic ratio. These cells often have abundant cytoplasm that sometimes is vacuolated. Phagocytosis of other cell types occurs with some forms of histiocytic sarcoma. Nuclei often have bizarre shapes and there often are multinucleated cells and bizarre mitotic figures. Definitive diagnosis is by histopathology and immunohistochemistry.

Neoplastic cells in metastatic carcinoma often occur in clusters and typically display cytologic features typical of the primary neoplasm, although these features may not help in identifying the cell of origin. Metastasis from tumors involving the pancreas and gastrointestinal tract are the most common metastatic carcinomas. Hemangiosarcoma is the most common sarcoma involving the liver. The neoplastic cells often occur individually or in small aggregates. The cells typically are very large, spindle-shaped cells with round to oval nuclei, fine chromatin, single or multiple nucleoli, and moderate to abundant basophilic cytoplasm. Rarely, formation of a vascular lumen-like structure can be identified on cytology. Gastrointestinal stromal tumors derived from the interstitial cells of Cajal in the gastrointestinal tract can metastasize to the liver. These cells have a very characteristic long, narrow, spindle-cell shape with very narrow elongated nuclei and minimal variation in cell size, nuclear size, and N:C ratio.

**SUGGESTED READING**
