Cytology of Internal Organs  
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With the use of ultrasonography, cytologic examination of abnormal findings is more readily performed on internal organs within the abdomen primarily but also the lung. Knowing the cytologic appearance of normal tissue is necessary in order to be sure the appropriate tissue has been sampled. During exploratory surgery, samples from incision or needle cutting instruments should also be prepared initially as a cytologic sample before placing the tissue into formalin for histopathology. Multiple slides should be made and preferably from areas of live tissue avoiding the centers of large lesions that may be necrotic. It is also important to recognize artifacts associated with this procedure that is not encountered from peripheral tissues. One major artifact found in cytologic preparations is the presence of ultrasound gel on the slide. This amorphous bright magenta material when abundant can hamper viewing of cellular elements. Recommendations are given to prevent this by using alcohol to cleanse the area just prior to sampling. Other concerns include excessive bleeding so coagulation panels should be conducted before sampling.

Liver

Indications

Hepatomegaly is the primary reason for liver aspiration. Other indications include the suspicion of neoplasia or inflammation within the liver. Nodular lesions can also be aspirated with the aid of imaging procedures such as ultrasonography. The only contraindications are abnormal hemostasis, caused by either thrombocytopenia (<30,000/µl) or decreased coagulation factor activity, and the suspicion of hemangiosarcoma, which might rupture if lacerated during aspiration.

Interpretation of liver aspirates

Normal

Normal hepatocytes are uniform, large, round to oval cells with abundant basophilic, somewhat granular cytoplasm. Cells contain one or two round centrally located nucleus with a single prominent pale blue nucleolus (Figures 1 and 2). Normal hepatocytes often contain a small amount of dark blue-black to green granular pigment. This pigment may be a type of bile pigment or occasionally may be lipofuscin, which is a breakdown product of cell organelles. Hemosiderin may also be seen within hepatocytes and is usually a more golden brown to blue-green color. Hepatocytes may occur singly or in clusters. Very infrequently, rectangular crystalline clear inclusions may be seen in the nuclei of some hepatocytes and are of no known significance. Biliary epithelial cells may also be seen in normal aspirates and are small cells that are uniform in size with round nuclei and relatively small amount of pale blue ciliated cytoplasm (Figure 3). Other cells occasionally observed in small numbers in aspirates from normal livers include mast cells, macrophages (Kupffer cells), and lymphocytes and neutrophils. Mesothelial cells from the surface of the liver are frequently seen in liver aspirates, and should not be confused with neoplastic epithelial cells or biliary epithelium.

Cholestasis

In animals with cholestasis, increased amount of pigment may be observed within hepatocytes. Much more significantly, extracellular canicular casts of bile, representative of the biliary tree, may be seen between hepatocytes and are used cytologically as evidence of cholestasis (Figures 4 and 5).

Inflammation

Inflammation is evidenced by the presence of neutrophils, macrophages, lymphocytes or eosinophils. Inflammation may be suppurative (neutrophils) or non-suppurative (lymphocytes or macrophages).

Vacular change

Vacuoles within the cytoplasm of hepatocytes are usually due to hepatic lipidosis, increased glycogen content, or hydropic degeneration. The presence of clear round discrete vacuoles are usually suggestive of lipid; the vacuoles may vary in size from small to large ballooning vesicles that distend hepatocytes, pushing the nucleus to the side, making them appear similar to small adipocytes or macrophages (Figures 6 and 7). On the other hand, poorly delineated, foamy areas present peripherally in the cytoplasm are usually suggestive of increased glycogen content or hydropic degeneration (Figure 8 and 9). Special stains, such as PAS for glycogen or oil Red-O for lipid, may be helpful in differentiating the cause of vacuolar change. Hepatic lipidosis is more commonly seen in cats, whereas increased glycogen content and hydropic degeneration is more commonly seen in dogs. Steroid hepatopathy is a common cause of increased glycogen content in both dogs and cats.

Nodular hyperplasia

Nodular hyperplasia is common in old dogs and may appear similar to primary or metastatic neoplasia ultrasonographically. Hyperplastic hepatocytes from these nodules usually appear similar to normal hepatocytes. Cytologic abnormalities may include subtle increases in cell and nuclear size and slight variation in cell and nuclear size. Nuclear inclusions appear more commonly (Figure 10). More binucleate hepatocytes may be observed, but the cells usually do not have features of malignancy (Figure 11).
Extramedullary hematopoiesis (EMH)

EMH is commonly observed in liver aspirates of animals with increased hematopoiesis. Cells are those encountered in a bone marrow aspirate (erythroid and myeloid precursors and megakaryocytes). Progression of maturation is orderly, thus distinguishing EMH from neoplasia.

Copper-associated hepatopathy is rare as an inherited condition but has been recognized in Bedlington terriers, West Highland white terriers, and Doberman pinschers. Copper toxicity may occur as a result of liver disease. The copper is visible within the cytoplasm of hepatocytes and appears as pale green somewhat crystalline appearing granules (Figure 12).

Primary neoplasia

Hepatocellular carcinoma

Neoplastic hepatocytes often resemble normal hepatocytes to some extent, in that they usually have abundant basophilic cytoplasm. They usually have features of malignancy, however, such as multiple nucleoli, variation in nuclear size, increased nuclei: cytoplasmic ratio, and variation in cell size, but can appear very well differentiated. Malignant hepatocytes are usually distinguishable from hyperplastic hepatocytes (Figure 13). Dogs are more likely to develop hepatocellular carcinoma compared with cats.

Biliary carcinoma

Malignant bile duct epithelial cells are similar to any other malignant epithelial cell, with cytologic features of malignancy. Biliary cells are smaller than hepatocytes with less cytoplasm (Figure 14). This type of neoplasia is more common in cats than dogs.

Hemangiosarcoma

This may be a primary or secondary neoplasm with metastasis often from the spleen. Neoplastic cells are individualized, large basophilic cells with indistinct cytoplasmic borders. Nucleus is oval to round with coarse chromatin and prominent nucleoli (Figure 15). Circulation of ten displays frequent nucleated erythrocytes or mature red cells with acanthocytosis.

Neuroendocrine tumors or carcinoids are derived from the APUD (amine precursor uptake and decarboxylation) cells of the biliary system. The cells are similar to other neuroendocrine tumors and are usually quite fragile, thus numerous naked or bare nuclei are seen. Intact cells are small with round central nuclei. Chromatin is usually condensed, nucleoli are indistinct, and the cytoplasm is moderate to abundant and usually pale in color with occasional fine granules.

Lymphoma

Lymphoma is found as both a primary and metastatic neoplasm and is usually relatively easy to diagnose when lymphocytes are medium or large size. Liver aspirates containing large numbers of small lymphocytes are more difficult to interpret, as the lymphoid population may be representative of an inflammatory process or less commonly small cell lymphoma. Surgical biopsy and histopathology, as well as blood, bone marrow, lymph node cytology, and PCR should distinguish the two processes.

Metastatic liver neoplasia

Metastatic carcinoma and neuroendocrine and endocrine tumors may come from the pancreas and GI tract commonly. Metastatic sarcomas may involve hemangiosarcoma plus leiomyosarcoma or other spindle cell tumors.

Histiocytic sarcoma

Malignant histiocytes may involve dendritic cells and/or macrophages. Disseminated histiocytic sarcoma is characterized by the systemic proliferation of malignant dendritic cells which display minimal to moderate malignant features. Cells have abundant pale basophilic cytoplasm with single or binucleated forms. Malignant macrophages present as pleomorphic often multinucleated histiocytes with marked cellular atypia. These cells are associated with phagocytosis of erythrocytes and leukocytes. This severe and progressive disease is referred to as hemophagocytic histiocytic sarcoma.

Myeloid neoplasia

Neoplastic myeloid cells commonly infiltrate the liver. If they are somewhat differentiated, recognizable progranulocytes with pink cytoplasmic granules will aid in the diagnosis. If they are undifferentiated, special cytochemical stains or immunochemistry will be necessary to distinguish the origin and distinguish the blast cells from lymphoid precursors.

Mast cell neoplasia

Neoplastic mast cells present well-differentiated with numerous metachromatic granules or poorly-differentiated as round cells with few cytoplasmic granules.

Pancreas

The pancreas is usually only aspirated if abnormal tissue is detected by imaging. Normal pancreatic exocrine tissue appears as sheets of epithelial cells having a single round nucleus and moderate amounts of finely granular and basophilic. They often appear in acinar formation with minimal nuclear atypia.

Inflammation

Frequent nondegenerate or degenerate neutrophils are present with the latter associated with presence of bacterial infection. Other inflammatory cells such as macrophages and lymphocytes may be seen. Bacteria may be found within neutrophils to confirm sepsis (Figure 16).
Neoplasia
Malignancy of the exocrine pancreas (pancreatic adenocarcinoma) presents with moderate nuclear changes that include features such as anisocytosis, anisonucleoliosis, multinucleation, prominent nuclei, increased nucleocytoplasmic ratio, etc. Acinar formation and tight cytoplasmic junctions help confirm the glandular origin. Neoplasia of the endocrine pancreas involves secreting islet cells. Most common are those involving beta cell tumors, also called insulinomas (Figure 17). Dogs present with severe hypoglycemia. Cytologic samples are composed of a naked nuclei appearance having intact small round nuclei with occasional anisokaryosis against a background of lightly basophilic cytoplasm having variably sized punctate vacuoles. This tumor may be very small within the pancreas but metastasize with more apparent masses within the liver or regional lymph nodes.

Kidney
Cytologic evaluation is often related to enlargement or deformity of the kidney. Aspirates of abnormally small kidneys are usually non-diagnostic. Either palpation or ultrasound may be used to locate the kidneys and direct the needle. Normal kidney cells appear individual or multiple as small sheets of round or polygonal epithelium. The nucleus is round with moderately coarse chromatin and one nucleolus. Feline renal cells contain more frequent punctate lipid vacuoles within their abundant basophilic cytoplasm compared with dogs (Figure 18). Due to the high vascularity of the organ, hemodilution is common.

Neoplasia
Neoplastic kidneys may be primary presenting with malignant renal epithelium (renal carcinoma) in adult animals and with malignant embryonic tissue in young animals (nephroblastoma). Metastatic neoplastic cells in the kidney are often associated with lymphoma. Renal tubular epithelial cells are usually present in the sample.

Adrenal gland
Similar to the pancreas, the adrenal gland is only evaluated when enlarged. Cortical cells are large with distinct cytoplasmic borders and the lightly eosinophilic to moderately basophilic abundant cytoplasm with frequent variably sized punctate vacuoles. Aspiration of these masses is best accomplished using ultrasonography.

Hyperplasia and neoplasia
Adrenal gland tumors may arise from the adrenal cortex or the medulla. Tumors of the cortex usually produce excess glucocorticosteroids, and result in hyperadrenocorticism. Tumors of the medulla produce excess catecholamines. The cells from adrenal cortical adenomas are similar to normal or hyperplastic secretory cells of the adrenal gland. They are also similar to aspirates of other endocrine organs, with most cells appearing as naked nuclei. The cytoplasm is usually moderately basophilic and often contains numerous small discrete vacuoles. Nuclei are round, uniform, and contain usually contain a single prominent nucleolus. Cells from adenocarcinomas may be more variable in size, with more prominent multiple nuclei. However, cells may also appear similar to cells from adenomas. Tumors of the adrenal medulla (pheochromocytomas or chromaffin cell tumors) are infrequent. Cells from these tumors are similar to those from other neuroendocrine tumors and consist of clusters of fairly uniform cells with numerous naked nuclei (Figure 19). The cytoplasm is light blue and may contain pale basophilic granules. The nuclei are round with a single small nucleolus and may display mild anisokaryosis. Significant criteria of malignancy are usually not present.

Lung
Indications to evaluate cytologically the lung often involve imaging abnormalities with distinct masses or increased interstitial density. The low density of the lung is normally associated with scant epithelium upon aspiration. The lung can be sampled by bronchoalveolar wash as well as by aspiration but only aspiration will be discussed her. The normal cell aspirated is usually the alveolar macrophage with cuboidal epithelium arising from bronchial structures. Sheets of mesothelium may be present (Figure 21). Blood vessels may be present along with hemodilution.

Inflammation and neoplasia
Changes with inflammation are similar to other tissues with the presence of inflammatory cells including neutrophils, eosinophils, macrophages, and lymphocytes. Evidence of infectious agents such as bacteria, protozoa, and fungi should be sought. Primary lung neoplasia appears as tight balls of large cells, often deeply basophilic with or without pale secretory epithelium. Malignant features in carcinoma are usually quite pronounced (Figure 22). Many carcinomas metastasize to the lung and distinction between primary and metastatic carcinomas cannot be made on cytology.
Additional Detail
Figures. (All preparations are Wrights stained unless otherwise noted)

**Figure 1.** Normal hepatocytes. Note the prominent single nucleolus.

**Figure 2.** Normal hepatocytes. Green-black cytoplasmic granules are thought to be lipofuscin but bile may look similar.

**Figure 3.** Normal biliary epithelial cells. Note that they are columnar with basilar nuclei and ciliated apical area.

**Figure 4.** Liver aspirate from a dog, low magnification. The presence of the dark bile plugs between hepatocytes is representative of bile canaliculi and is indicative of cholestasis.
Figure 5. Liver aspirate from a dog with cholestasis, high magnification, showing bile plugs between adjacent cells (arrows).

Figure 6. Liver aspirate from a dog with mild to moderate lipidosis. Note the clear vacuoles (lipid) in the cytoplasm of the hepatocytes.

Figure 7. Liver aspirate from a cat with marked hepatic lipidosis. Note the large vacuolated resemble adipocytes or macrophages when completely infiltrated with lipid.

Figure 8. Liver aspirate from a dog with hyperadrenocortism. Note the clearing or rarefication at the periphery of the hepatocytes, consistent with glycogen deposition.

Figure 9. Liver aspirate from a dog at post mortem. Vacuolar change is related to hydropic degeneration. Note how the foaminess appears the same throughout the cell.
**Figure 10.** Liver aspirate from a dog with hyperplasia. Note the intranuclear inclusion (arrow). There are several binucleated hepatocytes reflecting regeneration.

**Figure 11.** Aspirate of a liver showing an example of nodular regeneration. Cytoplasm is more basophilic and binucleation is commonly found.

**Figure 12.** Liver aspirate from a dog with copper toxicity. Note the large pale granules within the cytoplasm. These often impart a light blue-green color which is refractile.

**Figure 13.** Liver aspirate from a dog with hepatocellular carcinoma. Note the pleomorphism despite the low nucleocytoplasmic ratio. Multinucleation is common.

**Figure 14.** Liver aspirate from a cat with biliary carcinoma demonstrating the small uniform cell characteristic of ductular epithelium.
Figure 15. Aspirate of hemangiosarcoma in the liver of a dog. The cell on the left is a typical malignant endothelial cell with large round nucleus, prominent nucleoli, and abundant basophilic cytoplasm with numerous punctate vacuoles.

Figure 16. Higher magnification as the same aspirate shown in Figure 15. Note the lymphoblast (large arrow), bacteria (small arrows) and neutrophil (arrowhead).

Figure 17. Pancreatic islet cell tumor. Note the naked nuclei appearance of the cells and minimal anisokaryosis. The cytoplasm is filled with punctate vacuoles reflecting the secretory function of these cells.

Figure 18. Kidney aspirate from a cat. Note the large vacuoles within the renal epithelial cells. (Courtesy of Mary Anna Thrall)
**Figure 19.** Pheochromocytoma demonstrating the appearance of naked nuclei and a uniform size of the nuclei. (Courtesy of Kristin Nunez)

**Figure 20.** Aspirate of an internal organ displaying a small sheet of benign and normal mesothelium. Note the two-dimensional appearance of interlocking cells.

**Figure 21.** Aspirate of an organ displaying a small sheet of benign and normal mesothelium. Note the two-dimensional appearance of interlocking cells.