

Newsletter

CYTOLOGY OF THE SPLEEN



SUMMARY

Fine needle aspiration (FNA) of the spleen may be helpful in the diagnostic work-up and development of a treatment plan for patients with suspected disease involving the spleen. 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. Discordance between cytology and other clinical findings should be pursued by histopathology or other diagnostic techniques.

Sample collection

Indications for FNA of the spleen for cytologic evaluation include generalized splenomegaly, presence of a mass or nodular lesions,

abnormal echogenicity, or suspicion of neoplasia or inflammation. Potential complications include hemorrhage and seeding of neoplastic cells along the needle tracts, but these are rare. Thrombocytopenia is viewed by some as a contraindication for a FNA of the spleen. 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. The animal is placed in lateral or dorsal recumbency, the area over the site is surgically prepared, and a 1-1.5 inch 21 or 22 gauge needle is used for sample collection. A non-aspiration technique may result in more cellular samples with less blood contamination, but an aspiration technique with a 6-12-ml syringe also can be used.

Normal Spleen

Splenic parenchyma comprises red pulp, white pulp, and blood vessels associated with trabeculae that extend from a thick, smooth muscle capsule. The red pulp consists of red blood cells within blood vessels, endothelial lined sinuses, and a reticular meshwork. The white pulp includes lymphatic nodules and periarterial lymphatic

sheaths. Macrophages occur throughout, but are prominent in the marginal zone surrounding periarterial lymphatic sheaths. FNA can be minimally to markedly cellular, and often are contaminated with blood. The lymphoid population includes predominantly small lymphocytes with variable numbers of intermediate and large lymphocytes. However, if a lymphoid follicle is aspirated, there may be a focal area in which the majority of the cells are large lymphocytes with moderate amounts of basophilic cytoplasm and nuclei with fine chromatin and prominent nucleoli. These should not be mistaken for neoplastic lymphocytes. In lymphoid neoplasia (see below), neoplastic lymphocytes usually are distributed throughout the smear.

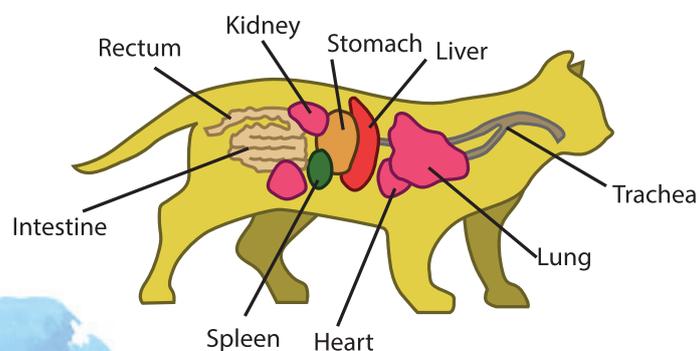
The number of macrophages is variable. Macrophages sometimes contain hemosiderin, which appears as bluish green to black pigment. Hemosiderin-laden macrophages often are prominent in aggregates of splenic stromal cells, which appear as dense aggregates of elongated cells amidst a variable amount of eosinophilic extracellular connective tissue. Occasional mast cells and plasma cells can be present in FNA from normal splenic tissue. Neutrophils often are from blood contamination.

Extramedullary hematopoiesis (EMH)

EMH occurs commonly in the spleen and is characterized by myeloid and erythroid precursors and megakaryocytes. EMH can be mild, moderate, or marked, depending on the cause. Maturation of these hematopoietic precursors should appear orderly, with fewer of the less mature stages and a predominance of the most mature stages of maturation.

Sidero-calcific plaques (siderotic plaques)

Firm plaques or nodules, often within the capsule, occur in some older dogs, likely from previous hemorrhage or hemolysis. FNA are characterized by hemosiderin or hematoidin either extracellular or within macrophages. Hemosiderin appears as bluish green to black irregularly shaped coarse granules, whereas hematoidin appears yellow, and often has a crystalline appearance. If there are collagenous fibers associated with calcium and hemosiderin, sometimes accompanied by granulomatous inflammation, these structures are called Gamna-Gandy bodies. Gamna-Gandy bodies can have a wavy or segmented like appearance, which could be confused with fungal hyphae. Special stains may be helpful in this distinction.



Hyperplasia

Splenic hyperplasia (reactive splenic tissue) can appear as diffuse splenomegaly or as nodules within the splenic parenchyma. Lymphoid hyperplasia is characterized by a mixed population of lymphocytes.

Although small lymphocytes typically predominate, there are increased numbers of intermediate and large lymphocytes, often in association with increased numbers of macrophages and plasma cells. Intermediate and large lymphocytes typically are less than 50% of the lymphoid population in lymphoid hyperplasia, in contrast to lymphoid neoplasia in which the majority of the lymphocytes are large and appear immature.

Aggregates of stromal cells are sometimes increased in splenic hyperplasia, and within these, there may be increased numbers of mast cells and abundant hemosiderin. Fibrohistiocytic nodules are firm, raised nodules containing macrophages, lymphocytes, plasma cells, and varying numbers of spindle-shaped cells that likely are fibroblasts. These may represent a continuum between nodular lymphoid hyperplasia and malignant mesenchymal tumors involving splenic stromal cells, but there is some controversy about this. It may be difficult to distinguish fibrohistiocytic nodules from malignant neoplasms based only on cytology; histopathology may be helpful in this distinction. Inflammation

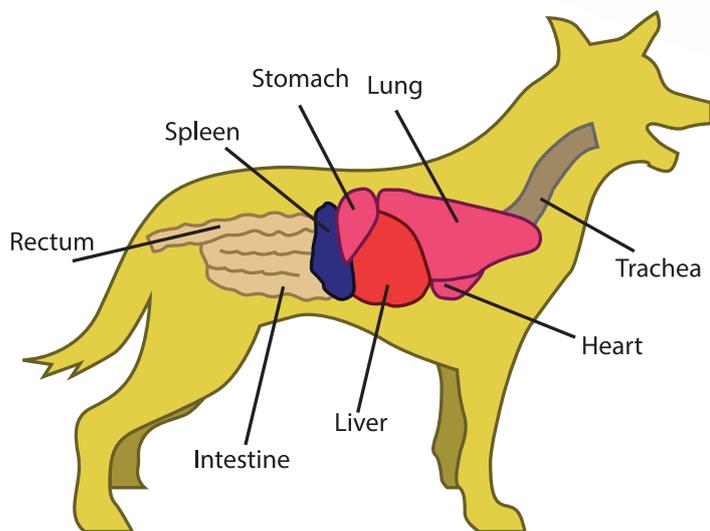
Macrophagic (histiocytic) inflammation occurs with some systemic fungal (e.g., histoplasmosis) and protozoal (e.g., leishmaniasis and cytauxzoonosis). Some dogs with immune-mediated hemolytic anemia and or thrombocytopenia have increased numbers of macrophages in the spleen. Necrotizing splenitis can be associated with increased numbers of neutrophils, eosinophils, and macrophages.

Neoplasia

Infiltration of the spleen with neoplastic lymphocytes can be part of multicentric lymphoma, hepatosplenic lymphoma, or chronic or acute lymphoid leukemia. In multicentric lymphoma, the neoplastic lymphocytes most commonly are large and have moderate amounts of basophilic cytoplasm. Nuclei are round, indented, or irregularly shaped and have fine to irregularly condensed chromatin and one to several prominent nucleoli. In some cases, the neoplastic lymphocytes are intermediate in size, have moderate amounts of moderately basophilic cytoplasm, smooth chromatin, and inconspicuous nucleoli.

Occasionally, the neoplastic lymphocytes are small and appear well-differentiated except for a small nuclear indentation. In these cases, immunophenotyping by flow cytometry may be helpful in supporting a diagnosis of either B-cell or T-cell lymphoma, and polymerase chain reaction for antigen receptor rearrangement (PARR) may be helpful to confirm that there is a clonal proliferation of lymphocytes.

In dogs with hepatosplenic lymphoma, neoplastic lymphocytes are restricted to the liver and spleen. Neoplastic lymphocytes are intermediate to large and have small to moderate amounts of pale to slightly basophilic cytoplasm that sometimes contains vacuoles or rarely, azurophilic granules. Occasionally, these cells are erythrophagocytic. Nuclei are round or cleaved and have fine to coarsely stippled



chromatin and prominent nucleoli. Binucleated and multinucleated cells may be present. There may be more variation in cell size, nuclear size, and nuclear to cytoplasmic ratio than is typical for other forms of high grade lymphoma. It may be difficult to differentiate this hepatosplenic lymphoma from histiocytic sarcoma based on cell morphology. The neoplastic cells in hepatosplenic lymphoma in dogs are positive for CD3 and in some dogs have been confirmed as $\gamma\delta$ T-cells. The lymphoma in these dogs can behave aggressively; bone marrow involvement may occur late in the disease.

Chronic lymphoid leukemia (CLL) can involve the spleen. There are usually numerous small lymphocytes that are present throughout the smear. These cells can resemble well-differentiated lymphocytes with nuclear indentations, or intermediate lymphocytes that sometimes contain azurophilic granules. The latter cells are called large granular lymphocytes (CGL) and are T-cell proliferations in dogs with CLL. A complete blood count (CBC) typically shows marked lymphocytosis due to lymphocytes with similar morphology. The rest of the CBC may be relatively normal. This is in contrast to acute lymphoid leukemia involving the spleen, in which case the neoplastic lymphocytes are large and appear immature, and the CBC often is characterized by marked lymphocytosis due to circulation of large lymphocytes, accompanied by non-regenerative anemia, neutropenia, and thrombocytopenia. Immunophenotyping by flow cytometry may be helpful for further evaluation of CLL and ALL.

Mast cell neoplasia can involve the spleen. In dogs, this can be from metastasis from a cutaneous mast cell tumor, in which case FNA of the spleen is part of staging the disease. In cats, mast cell neoplasia involving the spleen often is diffuse infiltration due to systemic mast cell neoplasia. The cytologic appearance of mast cells usually is very helpful in the diagnosis, but in some cases the granules do not stain very well. This is particularly true with some commercial stains. It is important to remember that normal splenic tissue contains mast cells, and these sometimes increase with inflammatory diseases involving the spleen. If there are aggregates of mast cells or the mast cells appear anaplastic, the index suspicion for mast cell neoplasia increases.

Histiocytic sarcoma involving the spleen 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.

Hemangiosarcoma is a neoplasm of endothelial cell origin and can involve the spleen. The neoplastic cells often occur individually or in small aggregates; very few neoplastic cells may be present on each individual smear.

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. Definitive diagnosis is by histopathology and immunohistochemistry.

Neoplastic cells from metastatic carcinoma or sarcoma can involve the spleen. These cells usually display cytologic features typical of the primary neoplasm, although these features may not help in identifying the cell of origin.

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