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Popular Article

Cytology of lymph nodes, spleen and thymus in commonly prevalent pathological conditions of companion animals

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Cytology is a simple, rapid, and widely used technique. Fine-needle aspiration biopsy or non-aspiration fine-needle biopsy procedures can be used to obtain samples for cytology from peripheral and internal lymph nodes or other lymphoid organs. Imprints or scrapings of tissues that are removed surgically or obtained during necropsy can also be used as samples for cytology. Cytology involves examining cells from various tissues and fluids for diagnosing the diseases. Cytology is essential for identifying cancerous cells, differentiating between benign and malignant tumors, detecting infections, and assessing inflammatory conditions. Furthermore, it provides rapid and minimally invasive diagnostic insights, thereby aiding veterinarians to provide timely and accurate diagnoses, leading to more effective treatment and improved outcomes in animals.

Lymph Nodes

In normal lymph nodes, lymphocytes accounts for 75-90% of the total cell population. These lymphocytes typically measure 7 to 10 µm in size, which is roughly 1 to 1.5 times the size of red blood cells. Their nuclei are usually spherical to oval in shape and is surrounded by a thin border of cytoplasm. Their nuclei contain dense clumps of dark chromatin and lack a visible nucleolus. Normal lymph nodes also contain a small proportion of intermediate (medium) lymphocytes, that makes up around 5-10% of the cell population and have diameter of approximately 9 to 15 µm. Additionally, less than 5% of the cell population in normal nodes consists of lymphoblasts, which typically have more than 15 µm diameter, roughly 2 to 5 times the size of red blood cells and appears larger than neutrophils. Lymphoblasts have moderate amount of basophilic cytoplasm, that appears granular due to dark-staining protein-rich areas and lighter-staining regions associated with some organelles. The nuclear shape of lymphoblasts varies, ranging from round to irregular, and often displays a stippled chromatin pattern. Single to multiple nucleoli are commonly found within lymphoblasts. In normal

lymph nodes, small numbers of plasma cells, macrophages, neutrophils, and mast cells can also be found but in very low quantities.

a) **Reactive Lymph Node or Reactive lymphoid hyperplasia:** In a reactive lymph node, small and well-differentiated lymphocytes form the predominant cell type but cats can have high number of intermediate lymphocytes and lymphoblasts. Lymphoblast population typically do not exceed 10 to 20% of the total lymphoid cells in a reactive node. Dogs have plasma cells in reactive lymph nodes. These plasma cells are round to oval in shape and of medium size, with a single round nucleus eccentrically located. The nucleus of a mature plasma cell has more cytoplasm but have same size and color as that of a small lymphocyte. The cytoplasm appears deeply basophilic and contains a visible golgi apparatus, which can be seen as a clear area located between the nucleus and the plasma membrane.

b) **Inflammation of the lymph node (lymphadenitis):** In cases of inflammation of lymph node, the main population of non-lymphoid inflammatory cells determines the type of inflammation. Suppurative inflammation is characterized by an increased number of neutrophils that surpass the expected number due to blood contamination. In this situation, more than 5% of nucleated cells are neutrophils. Typically, this arises from a bacterial infection either within the lymph node (resulting in an abscessed lymph node) or in the lymph node draining area. Eosinophilic inflammation is characterized by an inflammatory response that includes eosinophil infiltration, often accompanied by a slight increase in neutrophils. Allergic dermatitis is the most common cause of eosinophilic lymphadenitis that mainly affects the inguinal or popliteal lymph nodes. Other common causes include parasitic diseases, non-dermatologic allergic/hypersensitivity reactions, eosinophilic granuloma complex, eosinophilic gastroenteritis, mast cell tumors, and hypereosinophilic syndrome. Pyogranulomatous inflammation involves a significant macrophage component, either with or without the presence of neutrophils. This type of inflammation usually results from fungal infections like coccidioidomycosis, cryptococcosis, blastomycosis, and sporotrichosis, protozoal infections including toxoplasmosis, cytauxzoonosis, or leishmaniasis, mycobacterial infections, *Nocardia*/*Actinomyces*, and *Bartonella* infections in dogs.

c) **Lymphoid neoplasia (lymphoma):** Lymphoma is typically suspected when lymphoblasts makes up around 30% of the cell population in a lymph node aspirate, although the normal lymphoid population usually ranges from 50% to 90%. When more than 50% of lymphoblast cells are present, cytological diagnosis of lymphoma can be reliably established. Lymphomas can be categorized based on their tissue of origin e.g., renal, thymic, intestinal, etc. Multicentric lymphoma is most commonly seen in dogs. However, determining the "cytologic type" of lymphoma offers insight into the degree of malignancy, the likelihood of responding to chemotherapy, and the potential causes of paraneoplastic syndromes such as hypercalcemia.

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The most accurate method for typing lymphoma involves the use of lymphocyte markers to identify the specific subset of lymphocytes involved in the neoplastic process, such as T-cells like CD4 or CD8, B-cells, or Natural Killer cells.

d) **Metastatic disease:** To know about specific lymph nodes draining a particular area is crucial for detecting metastatic disease. However, the absence of obvious metastatic disease in a cytology does not rule out the potential for early metastasis because many tumors can enter the nodes through afferent or subcapsular vessels or can start as focal accumulations. Metastatic disease is characterized by the presence of a uniform cell population that is not typically found in a lymph node. These cells often appear anaplastic and exhibit clear signs of malignancy. While the remaining lymphoid population may seem reactive, the neoplastic cells can completely replace the lymph node parenchyma, therefore making cytologically identification of lymph node hard. The absence of lymph node enlargement does not necessarily rule out the existence of metastatic disease. Some neoplastic processes, like mast cell tumors, are known to metastasize without causing lymph node enlargement. Conversely, the presence of swollen lymph nodes in an area draining a tumor does not indicate that metastasis has occurred. Lymph nodes that drain regions with tumors often become reactive due to the regional inflammatory response triggered by the neoplasm.

Spleen

In aspiration samples taken from the spleen, the cellular components consist of splenic blood, stroma (referred as red pulp), and lymphoid tissue (known as white pulp). A splenic aspirate sample will inevitably contain a significant amount of blood due to the high hematocrit levels (80% to 90%) in the spleen. The white pulp of the spleen appears clearly defined as a variable number of lymphocytes scattered throughout the smear. When there is a predominance of lymphocytes in an otherwise blood-filled smear, it suggests the presence of splenic tissue. A typical lymphoid population consists mainly of small lymphocytes, with moderate numbers of intermediate lymphocytes, and variable but usually low numbers of plasma cells and lymphoblasts.

a. **Splenic hyperplasia:** Splenic hyperplasia refers to the proliferation of the spleen's normal cellular components, which occurs in response to cytokines, antigenic stimulation, and angiogenic factors triggered by various inflammatory, reactive, and neoplastic conditions. Hyperplastic lesions can manifest as either generalized or nodular and may predominantly affect the red pulp (characterized by histiocytic and stromal hyperplasia), the white pulp (exhibiting lymphoid hyperplasia), or both regions simultaneously. In cases of generalized hyperplasia, the cellularity of a splenic aspirate typically shows a high count, with numerous hypercellular stromal clusters. Alongside increased reactive lymphocytes and plasma cells, elevated numbers of macrophages, mast cells, stromal cells, and capillaries are also observed. Eosinophils may also be present in higher quantities, and reactive spleens often show

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significant increase in hemosiderin, with large dark clumps of iron associated with stromal clusters. Occasionally, it is necessary to assess peripheral blood and other hematologic organs to distinguish marked benign hyperplasia from neoplastic conditions involving lymphoid, histiocytic, or stromal elements.

b. **Splenic inflammation:** Both septic and non-septic inflammation can affect the spleen. The spleen is frequently implicated in systemic or multicentric inflammatory conditions due to its significant role in phagocytosis and immune regulation. In some cases of inflammation, necrosis can also be observed. Eosinophilic inflammation occurs in variety of situations, including infectious conditions like fungal infections, reactive responses, neoplastic disorders (like T-cell lymphoma), and immunologic diseases, including hypersensitivities and hypereosinophilic syndromes. However, infectious causes of inflammation are more common than non-septic causes. In most cases, the causative agents can be readily identified through examination of splenic aspirates. Examples of pathogens that can affect the spleen in dogs and cats include bacteria (e.g., tularaemia, salmonellosis), fungi, protozoa (e.g., cytauxzoonosis, leishmaniasis), and systemic yeast (e.g., histoplasmosis). The type of inflammation (purulent, granulomatous, mixed) and the characteristics of the inflammatory cells depends up on several factors like the type of organism involved, the extent of necrosis, the production of toxins, and the duration of the inflammation.

c. **Splenic neoplasia:** Neoplastic conditions affecting the spleen includes hemic neoplasia, mesenchymal neoplasia (involving non-hemic connective tissues), and, less frequently, metastatic carcinoma. Hemic neoplasia is often diagnosed through cytological examination. The absence of neoplastic cells in splenic aspirate does not exclude the possibility of neoplasia, particularly in cases involving connective tissue. Additionally, haemorrhage, necrosis, and extramedullary hematopoiesis are frequently associated with neoplastic conditions and complicate the diagnostic process. Hemic neoplasia is the most commonly reported splenic disorder in cats.

d. **Hemosiderosis:** Splenic hemosiderosis is the increased deposition of iron in the spleen occurring due to excessive erythrophagocytosis and the breakdown of haemoglobin. It can be observed in both benign and malignant splenic conditions. Hemosiderosis can occur from hemolysis due to extravascular destruction of red blood cells, or it may result from bleeding associated with conditions like haemangioma, hemangiosarcoma and hematoma. In splenic aspirates, hemosiderin is visible within macrophages as dark blue-black granular pigment. Large golden brown or black clumps of hemosiderin may be seen outside the cells and are often mixed with the clusters of splenic tissue. The distribution of macrophages containing hemosiderin is commonly diffuse, although in older dogs, focal siderotic plaques may develop

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beneath the splenic capsule. Prussian blue stain is frequently used to assess the distribution and severity of hemosiderin in the spleen.

Thymus

Thymus consists of two distinct cell populations: lymphocytes and reticular epithelium. The cell composition of the cortex resembles that of a lymph node, primarily consisting of small, densely staining lymphocytes, with occasional mast cells present. Among these small lymphocytes, large stellate cells having round vesicular nuclei form dense aggregations, and are referred to as Hassall's corpuscles. These compact clusters of the epithelial cells appear similar to epithelioid macrophages, characterized by abundant pale-blue cytoplasm and cellular connections between them.

a. **Thymoma:** Thymomas are the tumours that originates from thymic epithelial cells. They are most commonly found in older dogs with few cases reported in cats. They are typically located in the cranial mediastinum but can develop in any area through which thymic progenitor cells pass during embryonic development, such as the cervical region or the pericardial sac. Cytologically, thymomas consist of three main components: (i) A mixed population of predominantly small lymphocytes. (ii) A small number of loosely aggregated epithelial cells. (iii) Well-differentiated mast cells. The individual thymic epithelial cells can vary in size and shape, ranging from round and oval to polyhedral or spindle-shaped. They have round to oval nuclei, indistinct nucleoli, moderate amount of basophilic cytoplasm with unclear cell boundaries. In cases of malignant thymomas, there may be overtly malignant epithelial cells with increased nuclear-to-cytoplasmic ratios, cytoplasmic basophilia, anisocytosis, and anisokaryosis. Sometimes, malignant forms can also be present without significant cytological abnormalities.

b. **Thymic Lymphoma:** Thymic lymphoma is most commonly observed in dogs, young cats between the age of 6 to 18 months are positive for feline leukaemia virus with over 85% of cases located in the mediastinum. These lymphomas originate from T lymphocytes and involve the clonal expansion of large lymphocytes, which can be readily identified through cytological examination. However, there are also cases with small cell and mixed cell lymphomas, which may require additional diagnostic methods like flow cytometry and PCR for Antigen Receptor Rearrangements (PARR) testing for confirmation. In dogs, mediastinal lymphoma primarily manifests as a CD4+ disease.

Conclusion

In conclusion, cytology serves as a rapid and effective diagnostic tool for evaluating various conditions in lymph nodes, spleen, and the thymus. It allows the identification of normal cellular components and their alterations, including reactive lymph nodes, inflammatory responses, lymphoid neoplasia like lymphoma, and metastatic disease. In the spleen, cytology aids in detecting hyperplasia,

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inflammation, neoplasia, and hemosiderosis. Further, it plays a crucial role in diagnosing thymic disorders such as thymoma and thymic lymphoma. Overall, cytology provides valuable insights into these tissues, thereby facilitating early diagnosis and appropriate management of a wide range of pathological conditions.

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