

COMMISSIONED PAPER

Clinical Cytology of Companion Animals:

Part 3. Cytology of the Lymph Node

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INTRODUCTION

Of all indications for FNAB, the enlarged lymph node is the most rewarding. In general the peripheral lymph nodes are easy to fix with one hand and therefore easy to aspirate, the biopsy procedure is not painful, and the number of possible diagnoses is not high. It is thus not surprising that the history of the cytology of lymph nodes goes back to the beginning of this century. In 1904 Greig and Gray used this technique to demonstrate trypanosomes and after a publication by Guthrie in 1921, systematic diseases such as lymphomas were also diagnosed in this way. The cytologist who examines the FNAB of a lymph node should know whether this node was of normal size, possibly enlarged, or undoubtedly enlarged. A slight enlargement of the lymph node is difficult to confirm clinically. If the enlargement is dubious and no abnormalities are found cytologically, then the obvious conclusion is that the lymph node is normal. If the lymph node is unquestionably enlarged, then there must be a cause. The cytologist will then be more careful in giving a negative report and will advise follow-up histological examination. It can be very difficult to differentiate the cytological appearance of a normal lymph node from that of some well-differentiated lymphomas. In addition, the cytologist should know whether the FNAB was performed in order to answer a specific question. If the patient is suspected of having leishmaniasis or tumour metastases, then a longer and more specific search will be made for parasites or for tumour cells. For the same reason it is important to know whether the lymph node enlargement is generalized or concerns only one node and, in the latter case, whether there is (or has been) a tumour in the area drained by this node. It is also important to know which lymph node has been aspirated. The mandibular lymph nodes are often reactive, because many animals have inflammatory processes in the mouth. If there is generalized enlargement of lymph nodes, the mandibular nodes are therefore not the most suitable for cytological examination. Finally, it is important for the cytologist to know what therapy has already been given. The administration of corticosteroids causes lymphocytolysis and suppression of the immune response. This can greatly distort the cytological appearance of the nodes.

Normal appearance and benign changes

Although the normal lymph node is seldom aspirated, familiarity with the normal cytological appearance is necessary in order to recognize abnormalities. Mild antigenic stimulation also takes place in the normal lymph node and in principle all of the stages of B- and T-lymphocytes can be found. However, the majority

(85-95%) of the cells are small B- and T-lymphocytes. These cells are characterized by little cytoplasm, round nuclei without nucleoli, and often slightly rough chromatin structure (Fig. 1). The size of these cells (about 10µm) lies between that of erythrocytes and polymorphonuclear granulocytes.

The cytoplasm of lymphocytes is rather fragile and can be found in loose fragments throughout the smear, the so-called

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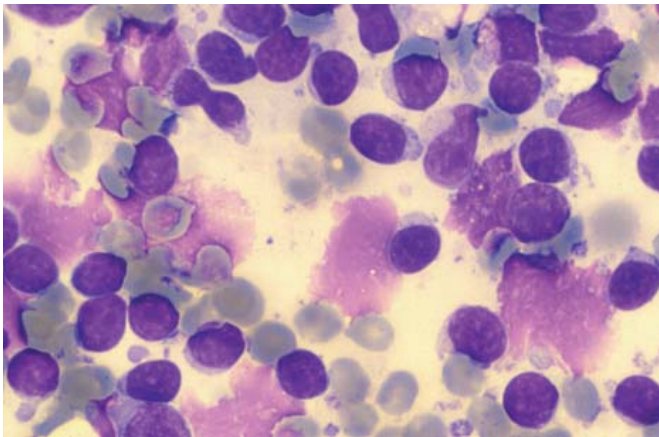


Fig. 1 FNAB of a normal lymph node of a dog. Monomorphic population small lymphocytes. Pink material in between cells is nuclear material of streaked out damaged cells. Small blue fragments are lymph glandular bodies.

lymphoglandular bodies (Fig. 1). With the May-Grünwald Giemsa stain they are light blue. Lymphoglandular bodies are characteristic of lymphoid tissue and their presence can be useful in differentiating lymphoid cells from those of an undifferentiated small cell carcinoma.

A normal lymph node also contains other developing stages of the lymphoid series but never more than 5-10% of the total number of cells. Other, nonlymphoid cells occurring in a normal lymph node include polymorphonuclear neutrophilic and eosinophilic granulocytes, macrophages, histiocytes, mast cells, erythrocytes, and monocytes. These cells are only present sporadically. An overview of the different types of cells in the normal lymph node is given in Table 1.

Non lymphoid tissue

The most frequent cause of the erroneous diagnosis of an enlargement of the mandibular lymph node is the mistaken palpation of a mandibular salivary gland, whether normal or enlarged (Fig. 2). In the dog and the cat the mandibular lymph nodes are rostral to the salivary gland and both are in principle readily palpated. Salivary gland cells are much larger than lymphoid cells, contain more cytoplasm, and form acinar (gland-

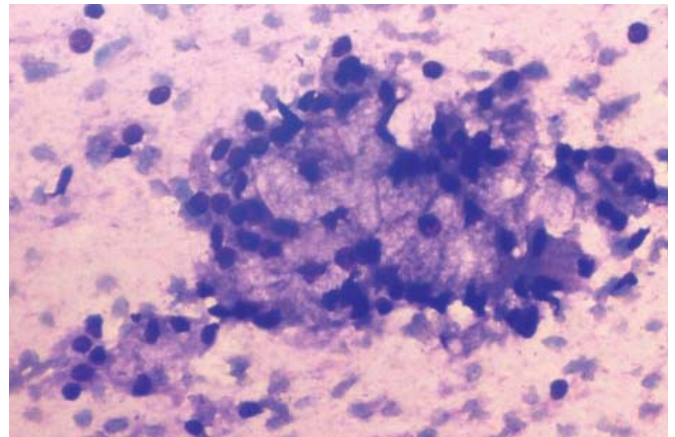


Fig. 2 Cluster epithelial cells in acinic structure with a mucoid background. Normal salivary gland tissue.

shaped) structures. Lymphocytes and lymphoglandular bodies are absent.

In obese animals it is possible to have the wrong impression that the lymph node is enlarged because it is surrounded by a thick layer of fat. An aspiration biopsy obtains mainly fat. It should be realized, however, that most fat tissue is dissolved in the fixation in alcohol, which is used in most staining methods.

Reactive hyperplasia

The most frequent cause of a generalized lymphadenopathy is reactive hyperplasia, via which the lymph node reacts to an antigenic stimulus. This can be the result of a viral, bacterial, or parasitic infection, or a reaction to tumour antigens, a foreign body, a skin disorder, or waste products of inflammation somewhere else.

Reactive hyperplasia is characterized cytologically by an increase in the number of large blast cells, such as immunoblasts and centroblasts, in relation to the number of small, normal

Fig. 3 Cell pattern of a reactive lymph node of a dog. A mixed lymphoid cell population is seen with several mature lymphocytes, some blast cells and one plasma cell (top left). A long naked nucleus of a histiocytic (epithelioid) cell is also present.

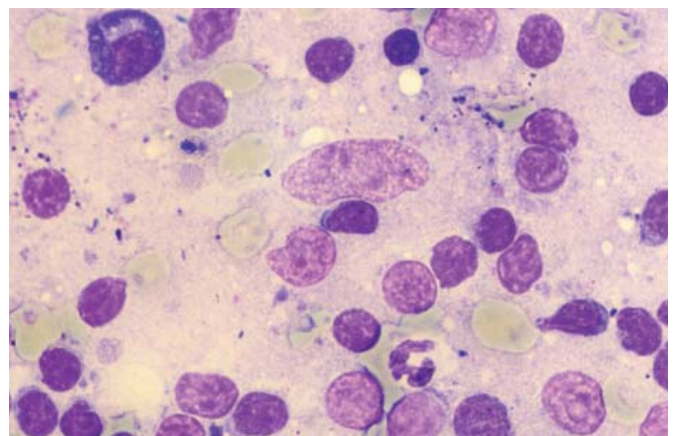


Table 1. Types of cells in a normal lymph node

cell type	frequency (%)
small, mature lymphocyte	80-90
prolymphocyte	5-10
young, blast cells	<5
plasma cells	0-5
eosinophilic granulocytes	0.3
neutrophilic granulocytes	0.1
mast cells	0.2
macrophages/histiocytes	0.4

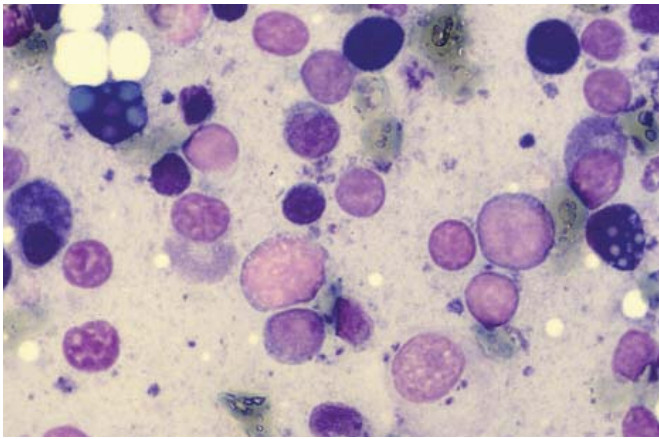


Fig. 4 FNAB of a reactive hyperplastic lymph node of a dog. Several plasma cells with Russell bodies are present.

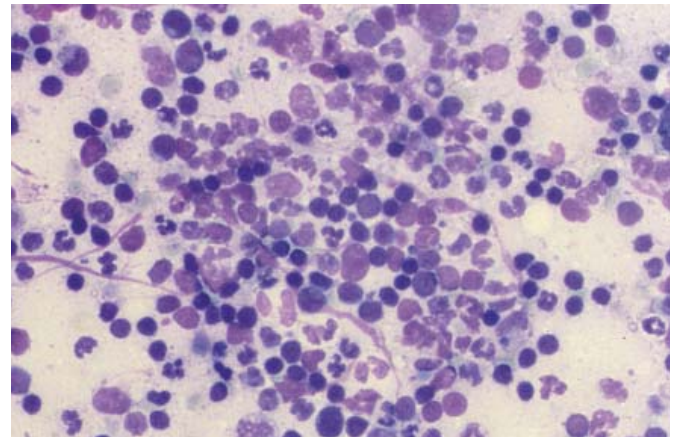


Fig. 5 Purulent lymphadenitis. Mixture of small lymphocytes and many neutrophils. In the background are some threads of degenerated nuclear material.

lymphocytes. There are also more mitoses and the number of lymphoplasmacytoid cells (intermediate stage between immunoblast and plasma cell) and plasma cells is increased (Fig. 3). Sometimes so-called "Russell's bodies" are seen in plasma cells (Mott cells) are seen in the cytoplasm of plasma cells. These are vacuoles filled with immunoglobulins (Fig. 4).

Depending on the cause of the stimulation there can also be increased numbers of other types of cells, such as macrophages, polymorphonuclear granulocytes and, especially in skin disorders, eosinophilic granulocytes and mast cells.

Lymphadenitis

The presence of many inflammatory cells in the lymph node is referred to as lymphadenitis. Differentiation into purulent and granulomatous lymphadenitis is made according to the types of inflammatory cells.

Purulent lymphadenitis is characterized by the occurrence of many polymorphonuclear granulocytes, usually combined with a light reactive lymphoid population and a few

macrophages. The difference from a reactive hyperplasia is sometimes difficult to confirm, but can also be quite clear, as in bacterial lymphadenitis. In the latter case there are many polymorphonuclear granulocytes, necrosis, and sometimes bacteria (Fig. 5). Lymphoid cells can even be completely absent. An increase in eosinophilic granulocytes is seen mainly in allergic dermatitis and such parasitic infections as leishmaniasis (Fig. 6). If bacteria are present they will be found in granulocytes, while parasites will mainly be found in macrophages.

Granulomatous lymphadenitis is also usually characterized by a slight reactive lymphoid picture and in addition an increase in macrophages, epithelioid cells, and multinucleated giant cells. Epithelioid cells are reticulum cells with an elongated oval nucleus, which is often indented and one end and which has a lightly granular chromatin pattern. Epithelioid cells are often missing cytoplasm in the preparation. Sometimes these cells occur in clusters and can then resemble carcinoma metastases. Granulomatous lymphadenitis is seen in toxoplasmosis, fungal and yeast infections, and certain bacterial infections (e.g., infections with *Mycobacterium* spp) (Fig. 7).

Fig. 6 Dog with leishmaniasis. The amastigotes are present in the large macrophage, but are also individually in the background of the smear. Several plasma cells are present.

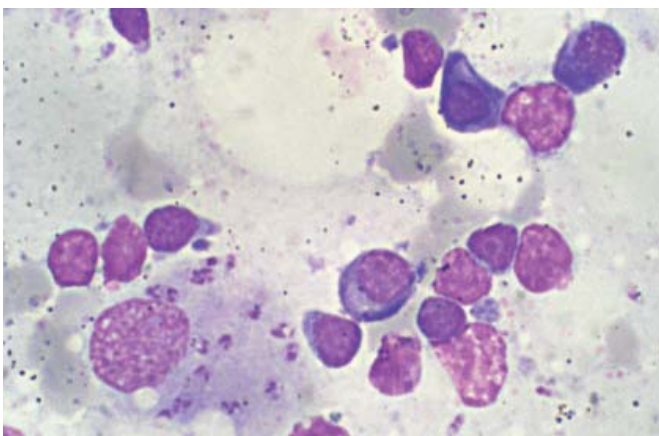
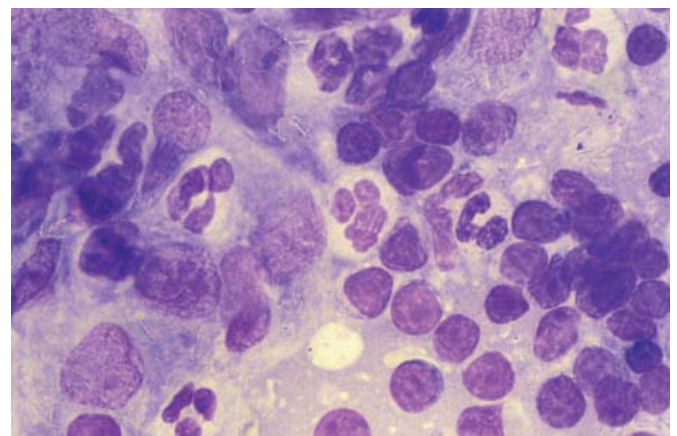


Fig. 7 In this FNAB of a granulomatous lymphadenitis due to a mycobacterium infection in a cat, several large histiocytic cells with some unstained rod bacteria can be seen on the left side of the picture.



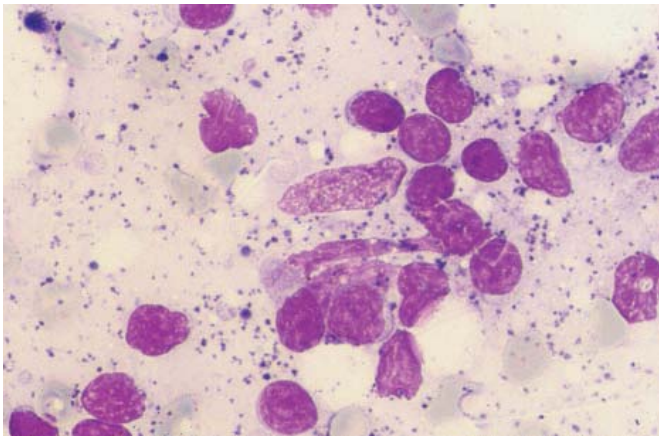


Fig. 8. Dermatopathic lymphadenopathy in a dog. There are several brown-black melanin granules in the background, several mature lymphocytes and a large nucleus of an interdigitating cell.

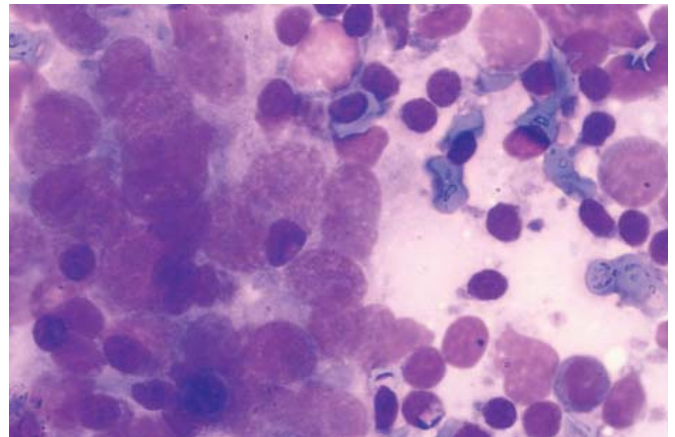


Fig. 9 Adenocarcinoma metastases of a mammary gland tumour in a prescapular lymph node in a dog.

A **dermatopathic lymphadenopathy** is a granulomatous lymphadenitis which occurs with skin disorders in which pruritus, scaling of skin and skin damage are prominent. The cellular picture is characterized by the presence of many brown-black melanin granules and a few eosinophilic granulocytes. Interdigitating cells are also encountered. These are elongated histiocytes with a reticular nucleus and a characteristic indentation of the nucleus (Fig. 8).

Malignant changes

Lymph nodes filter the lymph drained from a particular part of the body and remove the foreign material it contains. Tumour cells can also reach the regional lymph node in this way. In the lymph node the immune system can recognize the specific antigens expressed by the tumour cell and then eliminate the tumour cell. Sometimes tumour cells escape this "immune surveillance" and multiply themselves right in the lymph node. From here metastasis can occur to other parts of the body. In addition to metastases of tumour cells, the lymph node can become tumorous by neoplasia of cells of the hematopoietic system which normally occur in the lymph node. In this part further attention will be given to these two categories of malignancy involving the lymph node.

Metastatic malignancies

A complete survey of metastatic malignancies in the lymph node is not worthwhile, for in principle all malignant tumours can metastasize via the lymphatic system. Some types of tumours do metastasize earlier than others to the regional lymph node. Sarcomas generally spread earlier by hematogenous than by lymphogenous routes. Carcinomas, melanomas, and mast cells tumours are often found to metastasize to the lymph node, although this also depends upon the histological subtypes. In principle every cytological preparation from a lymph node in which there are cells that do not belong in a lymph node is suspect for metastatic malignancy. One must, however, take into account that when an aspiration is obtained from the lymph node some cells from the surrounding tissues can

be aspirated also. Hence it is always necessary to first evaluate the malignancy characteristics of the "foreign" cells, before speaking of malignancy. It is also possible that in one aspiration both the primary tumour and the lymph node are aspirated, via which a metastasis would be suggested. This can occur if a supramammary lymph node is biopsied simultaneously with the most caudal mammary gland, which contains tumour.

In addition to abnormal cells, the cytological appearance of a lymph node with metastases can also be characterized by benign changes as a result of the reaction of the immune system to the tumour cells. The number of macrophages, plasma cells, and young large lymphoid cells is often increased when there are metastases in the lymph node.

The cytological appearance of metastases in the lymph node depends very much on the histological type of the primary tumour. With an anaplastic carcinoma there are mainly separate cells of a type that does not belong to the lymph node and these can vary greatly in size. Mostly one encounters various cells that are many times greater than lymphoid cells and are easily recognized at low magnification. Many malignancy criteria, such as a variable N/C ratio, large nuclei, a high mitotic index, multiple and sometimes pathological nucleoli can be found. With more differentiated carcinomas, such as adenocarcinomas, cell clusters and sometimes even acinar structures are encountered (Fig. 9). Aggregates or syncytia of macrophages and epithelioid cells can resemble a cluster of metastasized carcinoma cells and must not be confused with them.

Another type of carcinoma that can usually be easily classified is the squamous cell carcinoma. With this tumour small clusters of small carcinoma cells with rather little, deep blue cytoplasm are encountered beside cells with a large amount of cytoplasm in different stages of keratinization. Keratinization is recognizable in the May-Grünwald Giemsa stain by the uniform sky blue color of the cytoplasm sometimes containing a few small "droplets". A characteristic difference from normal keratinized epithelial cells is that during keratinization the nucleus of the carcinoma cells does not degenerate, but remains present.

A few mast cells are always found in a normal lymph node or one that has benign changes. According to the literature, however, the number never exceeds 3%. More mast cells are suggestive of

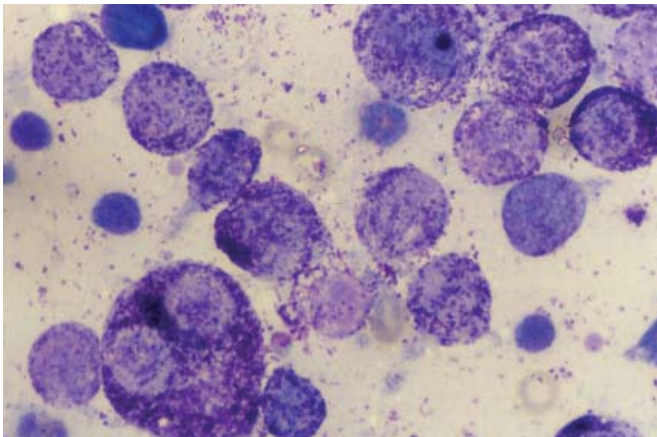


Fig. 10. Mast cell tumour metastases to a lymph node. Mast cells are numerous, differ in size, have prominent nucleoli and a binuclear cell can be seen.

a metastasized mast cell tumour or even mast cell leukemia. The mast cells can contain many purple-blue granules, sometimes so numerous that they obscure the nucleus. The cell can, however, also be sparsely granulated or even nongranulated (Fig. 10). In the latter case the mast cells are difficult to recognize as such. Another tumour that sometimes metastasizes to the lymph node is the malignant melanoma. This tumour cell is larger than the lymphoid cell, varies in form from round to spindle-shaped, and has a slightly oval nucleus. Bizarre nuclear shapes and multiple nuclei are frequently observed in malignant melanoma. The nucleolus is not always visible. The cell is usually characterized by the occurrence of black to brown-black granules in the cytoplasm. There are, however, also amelanotic melanomas. These contain few or no melanin granules and are thus difficult to identify. Melanophages can be confused with melanocytes. Melanophages are phagocytizing cells which have taken up pigment and occur in the lymph node in large numbers especially with skin disorders associated with pruritus and damage to the skin. Usually the cells are recognizable as such because the cytoplasm is slightly vacuolated and contains

Table 2. Simplified classification of lymphomas by cell type, based on the Kiel classification according to Lennert, 1974

Lymphocytic
Immunocytic
Plasmacytic
Centrocytic
Centroblastic/centrocytic
Centroblastic pure centroblastic anaplastic centrocytic polymorphic centroblastic
Lymphoblastic
Immunoblastic
Other histiocytic 'multilobated' cell

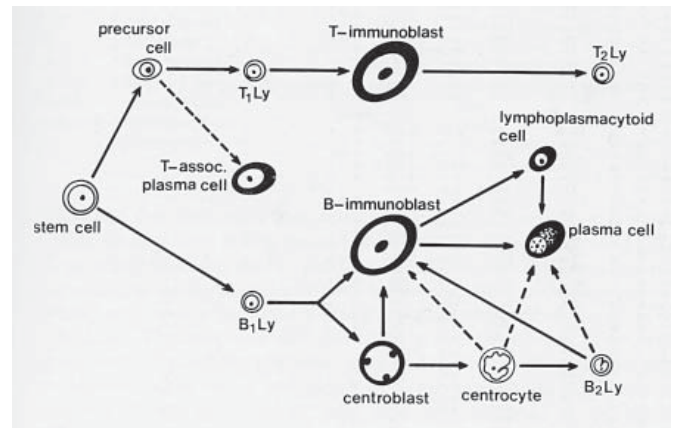


Fig. 11 Transformation scheme of normal lymphocytes (after Lennert) (from: Van Heerde (P.) - Malignant lymphomas and histiocytic tumours. Cytology and other diagnostic methods. Thesis, Uitg Rodophi, Amsterdam, 1984)

other phagocytized material besides melanin. In addition, they have no malignancy characteristics.

Lymphoid malignancies

Primary malignant transformation of the lymph node usually involves cells of the lymphoid system. Such cells as epithelioid cells and histiocytes are seldom involved. In the dog and cat these lymphoid tumours are called malignant lymphoma or lymphosarcoma. They are comparable to non-Hodgkin's lymphoma in man. Since Hodgkin's lymphomas have never been convincingly demonstrated in the dog and cat, the lymphoid tumours in these animals are usually simply called malignant lymphomas.

The cytological appearance of the malignant lymphoma can vary from patient to patient. One assumes that a lymphoid cell in each stage of its development can become malignant, whether by a blockage in further differentiation or by autonomous proliferation of a certain cell type (Fig. 11). The cell types which are encountered in malignant lymphoma thus do not differ in appearance from normal lymphoid cells. The cytological differentiation rests on the presence of a monotonous cell population, while in a non-lymphomatous lymph node all different development stages of the lymphoid series are visible. Various classifications schemes have been developed for non-Hodgkin's lymphomas in man. The Kiel classification (Lennert, 1974) is based entirely on the transformation scheme for normal lymphocytes described and is very suitable for cytological purposes (Table 2) (Fig. 12).

In the literature this Kiel classification has been applied successfully to malignant lymphomas of dogs. There is as yet no information about the applicability of this classification for malignant lymphomas of cats. A detailed description of the various types of lymphomas is presented below for readers who are interested. This is of interest for scientific objectives and to illustrate the diverse forms in which the malignant lymphoma occurs, but under practical conditions the diagnosis of malignant lymphoma and its subclassification into low- or high-grade

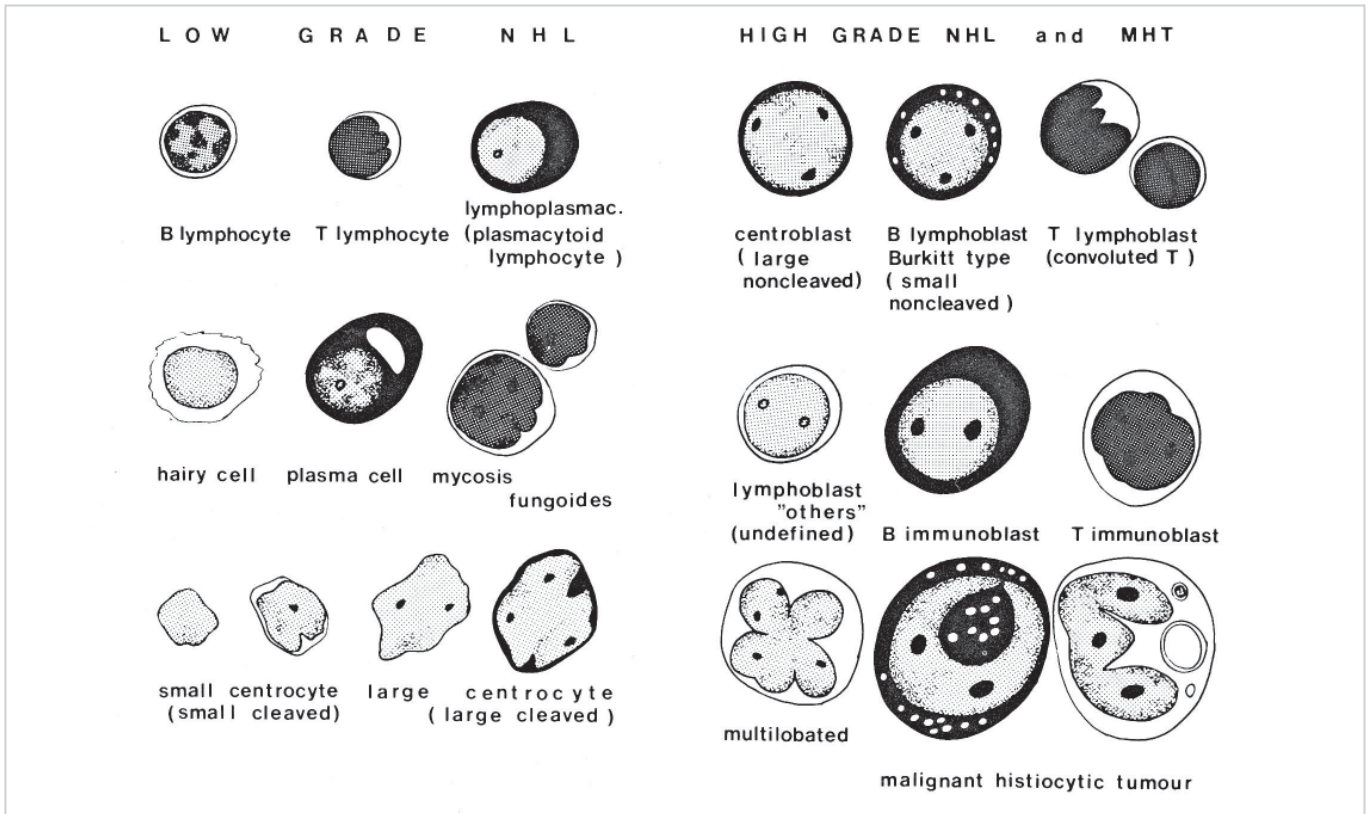
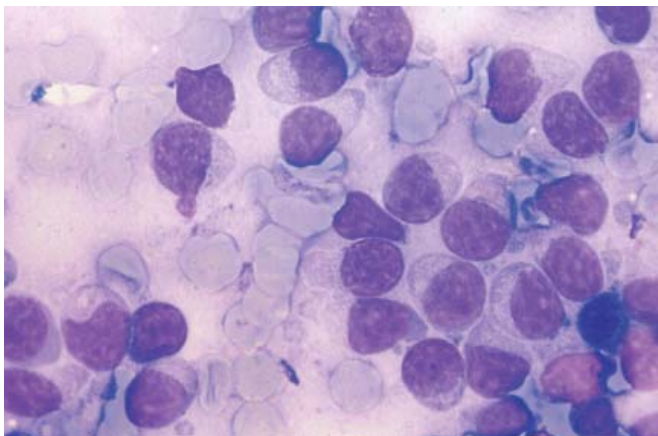


Fig. 12 Schematic drawing of malignant lymphoma cells according to Kiel classification (after Lennert) (from: Van Heerde (P.) - Malignant lymphomas and histiocytic tumours. Cytology and other diagnostic methods. Thesis, Uitg Rodophi, Amsterdam, 1984)

lymphoma is usually sufficient.

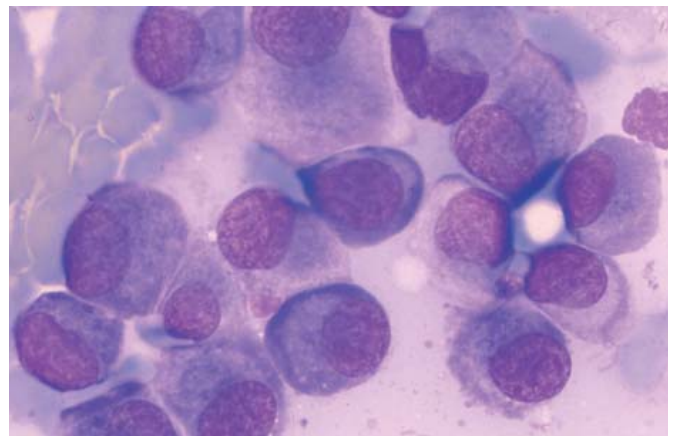
If the aspirated cell population consists mainly of characteristic blasts, the diagnosis of malignant lymphoma is not so difficult to make. However, there are also forms of lymphoma in which the tumour cells are difficult to differentiate from mature lymphocytes, especially for the less experienced cytologist. This can be the case, for example, with the lymphocytic and centrocytic lymphomas. Problems can also occur when the lymphoma contains more than one cell type, as in the immunocytic or centroblastic/centrocytic lymphoma. In many of these cases, the cell combination of enlarged lymph node and

Fig. 13 Lymphoplasmacytoid lymphoma. Several small lymphoid cells with abundant cytoplasm and a slightly eccentrically placed round nucleus.



nonreactive cellular appearance is decisive, especially if the cell population is monomorphic. If in doubtful cases there are a few more plasma cells or other inflammatory cells, more experienced help should be sought or the diagnosis should be confirmed by a surgical biopsy for histological examination. Sometimes the preparation consists almost entirely of individual degenerated nuclei and streaks of nuclear material. This can be the result of inexpert streaking out of the aspirate, but it can also be the result of necrosis. If such a preparation is carefully searched, particularly not neglecting the margins, then a location with intact cells may be found. Sometimes the majority of the loose nuclei in such a preparation contain definite remnants of

Fig. 14 Plasmacytic lymphoma. Large, atypical plasma cells.



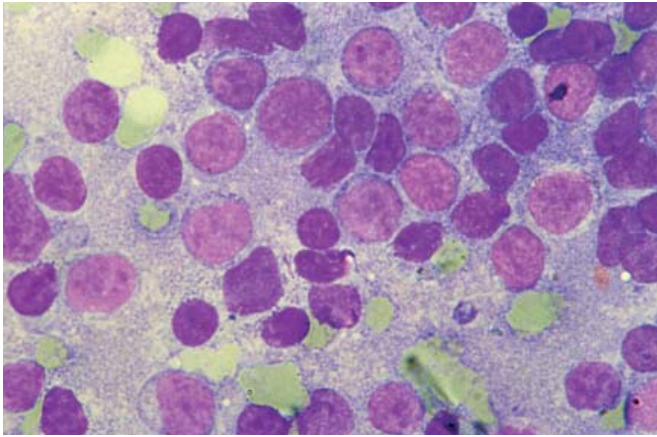


Fig. 15 Centroblastic/centrocytic lymphoma. A mixed population of large centroblasts, small centrocytes (cleaved cells), and lymphocytes.

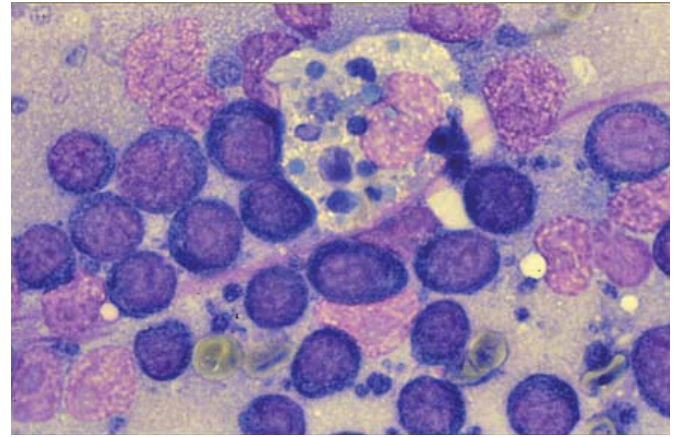


Fig. 16 (Polymorphic) centroblastic malignant lymphoma. A monomorphous populations of round centroblasts (with multiple small nucleoli). A large starry sky macrophage with cellular debris 'tingible bodies' is also present.

nucleoli. This is suggestive of a malignant lymphoma, but it is risky to base the diagnosis on this alone.

Morphology of canine lymphomas, classified by the Kiel classification

Lymphocytic lymphoma

This type of lymphoma consists of a monotonous population of small, mature lymphocytes. The cytological appearance is often difficult to differentiate from a nonreactive, normal lymph node. If this picture is found in a definitely enlarged lymph node without any evidence of reactivity, the chance is great that this type of lymphoma is present. If the lymphocytic lymphoma consists of B-lymphocytes, which can only be confirmed with certainty by immunotyping, then the nuclei are usually round and have a slightly rough chromatin pattern. This is in contrast to the T-lymphocytic lymphoma, in which the nucleus is slightly indented and has a dense chromatin pattern. Both types of lymphoma exhibit little cell multiplication. The lymphocytic lymphoma occurs infrequently in dogs.

Lymphoplasmacytoid / Immunocytic lymphoma

The most important cell type here is the immunocyte, a small lymphoid cell with more cytoplasm than the cells of a lymphocytic lymphoma and a slightly eccentrically placed round nucleus. This cell type has developed a little farther in the direction of the plasma cell. The lymphoma is called lymphoplasmacytoid lymphoma (Fig. 13). In addition to this cell type a few centrocytes, immunoblasts, and plasma cells can be found. The majority of the cells are, however, small lymphocytes. In this case the term immunocytic lymphoma is used.

Plasmacytic lymphoma

The occurrence of plasmacytoma in lymph nodes is extremely rare (Fig. 14). In this type of lymphoma mainly atypical plasma cells in diverse stages of development are found.

Centrocytic lymphoma

This lymphoma consists primarily of centrocytes. Centrocytes

are small cells with an irregular, sometimes indented nucleus. The cytoplasm is often absent or is very pale. The chromatin pattern is fine and there are usually no visible nucleoli.

Centroblastic/centrocytic lymphoma

As the name indicates, this type of lymphoma consists of both centrocytes and centroblasts (Fig. 15). Centroblasts have a large, round nucleus with multiple nucleoli that often lie adjacent to the nuclear membrane. The cytoplasm consists of a thin, dark blue rim. Many mitoses can be found. If the percentage of centroblasts is higher than 30-50%, the lymphoma is called centroblastic.

Centroblastic lymphoma

The most important cell type is the centroblast, but a few centrocytes will often be present. There are two special forms. If there are immunoblasts in addition to centroblasts, the tumour is called a 'polymorphic centroblastic' lymphoma (Fig. 16). If there are more than 50% immunoblasts, the tumour is considered to belong to the immunoblastic lymphomas (see below). The other special form is the 'anaplastic centrocytic' lymphoma. Anaplastic centrocytes are large centrocytes with a large, irregularly formed nucleus. The cytoplasm is often more lightly stained than that of the centroblasts. In the dog the centroblastic, polymorphic centroblastic, and anaplastic centrocytic lymphomas the most frequently occurring types of lymphoma.

Lymphoblastic lymphoma

The lymphoblastic lymphoma is infrequent in the dog. The lymphoblast is a medium-sized, round to oval cell with a thin rim of light to moderately basophilic cytoplasm that is sometimes vacuolated. The nucleus has a fine chromatin pattern with a few small nucleoli. Many mitotic figures can be present. In humans this type of lymphoma sometimes contains 'starry sky macrophages' (Fig. 16), which are thought to be characteristic of a certain subtype, the so-called 'Burkitt lymphoma'. In the dog, however, this type of macrophage (large, vacuolated macrophages that have phagocytized all kinds of material) are found in various types of lymphomas.

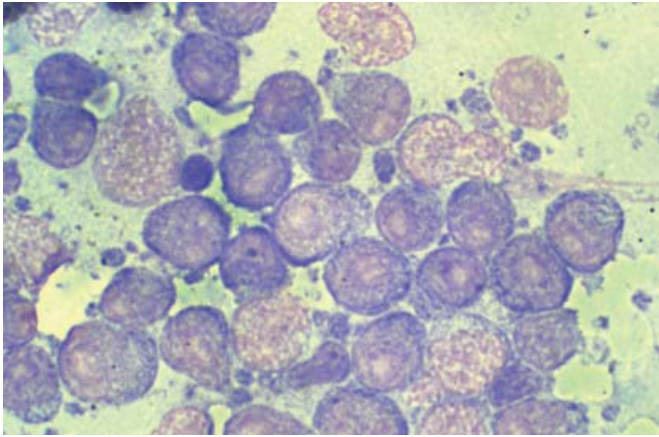


Fig. 17 Immunoblastic lymphoma in a dog. Apart from some naked nuclei a monomorphous population of immunoblasts is present, characterized by a large centrally located nucleolus.

Immunoblastic lymphoma

If at least 50% of the cells in a preparation are immunoblasts, a diagnosis of immunoblastic lymphoma is made (Fig. 17). Immunoblasts are large cells with a large, round, often eccentrically located nucleus. This nucleus is characterized by one large, centrally located nucleolus. Immunoblasts have a thick margin of blue cytoplasm. In addition to the large immunoblast with a slightly eccentrically located nucleus and much cytoplasm, in the dog there is also a smaller type of immunoblast with a smaller nucleus located centrally in the cell. The common characteristic with the large immunoblast is the large and centrally located nucleolus.

Other types

Other types of lymphoma that occur incidentally are mycosis fungoides, histiocytic lymphomas (Fig. 18), and multilobated lymphomas. In view of their low frequency of occurrence, they are not discussed in this overview.

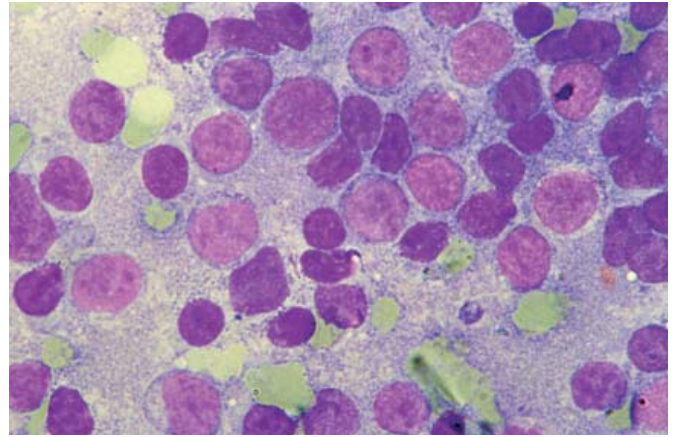


Fig. 18 FNAB of a lymph node with histiocytic sarcoma. Large cells with sometimes small cytoplasmic vacuoles and very irregular forms of nuclei. Phagocytosis and ring-shaped nuclei can also be present (not on this picture).

Suggested Literature

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