PRESENTATION AND DIAGNOSTIC APPROACH TO FELINE LYMPHOMA

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FRANCESCO CIAN DVM, DipECVCP, FRCPath, MRCVS describes occurrence, cause, locations in the animal, behaviour and diagnosis of lymphomas in cats

Summary

Lymphoma accounts for nearly one-third of all cancers in cats, with a prevalence that is higher than in all other species. Despite the high frequency, there is little information available about this feline disease, especially when compared with the canine species. This article will briefly outline the typical clinical presentation of feline lymphoma before focusing on its diagnostic approach.

LYMPHOMA is defined as a neoplastic disorder of the lymphoid tissue. It has been previously called lymphosarcoma or malignant lymphoma to underline its mesenchymal origin and malignant behaviour.

The development of lymphoma is not anatomically restricted and any nodal (for example, lymph nodes, spleen, thymus or mucosa-associated lymphoid tissue) and extranodal (for example, skin, eye, kidney or CNS sites can be affected).

Incidence and aetiology

Historical epidemiological investigations have estimated lymphoma accounts for nearly one-third of all feline tumours, with an annual incidence of 200 in 100,000 cats at risk (Dorn, 1967; Essex et al, 1976). The aetiology of lymphoma in domestic animals is not fully understood and is likely to be multifactorial; however, the relation between retroviral infections and lymphoma is universally recognised.

Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are the main viruses involved in the pathogenesis of lymphoma. FeLV has been demonstrated to be directly correlated to the development of lymphoma (mainly mediastinal forms) through an acquired insertional mutagenesis in which the integrated provirus may activate a proto-oncogene or disrupt a tumour suppressor gene (Fujino et al, 2008). On the other hand, the role of FIV in the development of lymphoma has not been completely clarified. The majority of published studies addressing this issue suggest oncogenesis arises via indirect mechanisms and is related to the immunosuppressive effects of the virus (Magden et al, 2011).

The underlying causes of non-retroviral-induced feline lymphoma are still unclear. Several factors have been considered to be related to the onset of lymphoma, including previous chronic inflammation (for example, irritable bowel disease, *Helicobacter* infection), environmental factors (such as exposure to tobacco smoke) and genetic predispositions (for example, Siamese cats; Louwerens et al, 2005).

Interestingly, several studies have shown the incidence of feline lymphoma has risen in past decades, with a change in incidence of the different forms. Before control of FeLV by serology testing and vaccinations in the early 1980s, more than 70 per cent of all cats with lymphoma were FeLV-positive and showed mediastinal involvement. After that, a decrease in these forms and an increase in the non-retroviral-associated lymphoma (mostly alimentary and extranodal) have been noted (Louwerens et al, 2005).

Pathology and natural behaviour

Lymphoma may be classified according to the location in the body, as mediastinal, alimentary, multicentric, nodal and extranodal. All these forms may secondarily involve bone marrow and/or peripheral blood and become leukaemic (leukaemic lymphoma).

Alimentary lymphoma

Alimentary lymphoma is the most common location for lymphoma in felines. It mostly occurs as a single lesion or diffuse thickening in the small intestine, but large intestine, mesenteric lymph nodes and the liver may also be affected. Cats with alimentary lymphoma are generally old and FeLV-negative; the Siamese breed appears to be over-represented. Clinical signs are variable and include decreased appetite, weight loss, diarrhoea, and vomiting (Gieger, 2011). Histologically, several subtypes have been identified. Cell type (large and small cells) and pattern of infiltration

(mucosal, transmural) appear to correlate with the clinical behaviour; small cell lymphoma with a mucosal superficial distribution have longer median survival times when compared with the large cell forms with a transmural pattern (Moore et al, 2012).

Another characteristic subtype of lymphoma called large granular lymphoma (LGL) may occur in the intestine of cats. This is characterised by a clonal proliferation of LGL lymphoid cells, which are easily identified on cytology since they contain multiple magenta or azurophilic granules of variable sizes (Figure 1).

LGL lymphoma has a poor prognosis and does not have a good response to chemotherapy. They frequently present with multiple organ involvement and may be leukaemic (Roccabianca et al, 2006).

Mediastinal lymphoma

The mediastinal form can involve thymus, mediastinal, and sternal lymph nodes. It is more commonly observed in young FeLV-positive cats. The majority of mediastinal lymphomas have a T-cell phenotype, since thymus is the primary haematopoietic organ where T-cells undergo maturation. Clinical signs are mostly related to the mass effect and include cough, dyspnoea and occasionally regurgitation and vomiting (Vail, 2012).

Multicentric lymphoma

Lymphoma confined to peripheral and/or internal lymph nodes is unusual in the feline species – representing less than 10 per cent of cases (Vail, 2012). An additional form of nodal lymphoma in cats is referred as T-cell rich B-cell lymphoma or Hodgkin's-like lymphoma. This form has a unique morphology, it selectively involves lymph nodes of the head and neck and is generally slowly progressive, as an indolent lymphoma (Walton et al, 2001).

Extranodal lymphoma

Together with the alimentary form extranodal lymphoma is the most common presentation of lymphoma in the feline species. Nasal cavity, kidney, and CNS are the most common extranodal locations. Extranodal lymphomas affect cats of variable age, usually FeLV-negative.

Nasal lymphoma is the most common extranodal location. It is usually localised and affects adult/old subjects, with a more favourable outcome when treated, compared with other anatomic forms of lymphoma. Large cell forms with B-cell phenotype are over-represented (Haney et al, 2009; Little L et al, 2007).

Renal lymphoma occurs in approximately one-third of extranodal cases. High-grade forms with B-cell phenotype are common. Extension to the CNS is a frequent sequela (Vail, 2012).

CNS lymphoma are the most common malignancies encountered in the nervous system and can be intracranial, spinal or both. It may be primary, but more commonly represents the extension of a multicentric process (Marioni-Henry et al, 2008).

Diagnostic approach to lymphoma

Clinical presentation of lymphoma in domestic animals is very variable and diagnosis is commonly based on the combination of clinical examination, diagnostic imaging and further laboratory testing. Cytopathologic and/or histopathologic evaluation of lymph nodes or involved organ tissues are always required for a definitive diagnosis.

Aspirates from lymphoma are generally highly cellular and are characterised by the presence of a monomorphic population of lymphoid cells, accounting for between 50 per cent to 100 per cent of all the nucleated cells present in the preparation (Figure 2). Several systems have been proposed in past decades to classify lymphoproliferative diseases in both humans and domestic animals. The updated Kiel classification is used in cytopathology for classification. This system takes into account elements including cell size, mitotic activity and immunophenotype (Table 1). Classification has an impact on the clinical side and has a prognostic value. Small cell lymphomas are commonly associated with a low mitotic activity, have a slow clinical progression and are poorly responsive to chemotherapy. Meanwhile, large cell lymphomas have a high mitotic activity, with a more aggressive clinical behaviour, but are generally more chemosensitive.

Diagnostic accuracy of cytology for diagnosing lymphoma is generally high, especially when the smear preparations are of good quality and examined by a specialist. However, in the feline species, more than in any other, several reactive processes can mimic lymphoma and make its diagnosis very challenging.

Lymphoid hyperplasia is described as a reactive process of the lymphoid tissue, after persistent non-specific antigenic stimulation. Cytologically, this is characterised by the presence of a heterogeneous and mixed population of lymphoid cells, with a prevalence of small lymphocytes and a variable, but lower, percentage of intermediate to large forms (15 per cent to 50 per cent of lymphoid cells). Other cell types, including plasma cells and mast cells, may also be present (Figure 3).

When the percentage of intermediate to large lymphoid cells is significantly increased and comes close to 50 per cent, the distinction from lymphoma may be difficult, and sometimes not possible.

Moreover, in the feline species, other specific reactive processes can be difficult to differentiate from lymphoma, such as the generalised lymphadenopathy resembling lymphoma and the distinctive peripheral lymph node hyperplasia (DPLH) of young cats. The latter is a self-limiting condition affecting mostly young cats, generally FeLV-positive, and causing generalised lymphadenopathy. Cytologically, it is characterised by an increased percentage of intermediate to

large lymphoid cells; histopathology may be necessary for a definitive diagnosis (Moore et al, 1986). Additional diagnostic techniques are warranted in cases where cytologic results were inconclusive or not definitive; histopathology significantly increases the probability of a final diagnosis and serves to further characterise the process, since it has the advantage of providing the architectural features of the affected tissue, on top of the cellular morphology.

Depending on the tissue targeted, different types of biopsies may be indicated. For alimentary lymphoma, full thickness samples are preferred to endoscopic biopsies for identification of lymphoma in deeper tissues (submucosal/muscolaris/serosal layers) and for evaluation of its distribution (mucosal, transmural), which has been reported to have a prognostic significance. However, this procedure is more invasive, implying an exploratory laparotomy, which is not always possible, especially when the patient is critically ill.

Additional diagnostic techniques (for example, flow cytometry and PCR for antigen receptor rearrangement; PARR) have been optimised for feline patients and may be helpful in challenging cases.

Flow cytometry

Flow cytometry is a diagnostic technique commonly used in veterinary oncology for the characterisation of lymphoma and leukaemia. It is a laser-based technology, which is based on the use of antibodies targeting specific surface markers (cluster of differentiation; CDs) expressed by lymphoid/myeloid cells, which help in the identification and proper characterisation of the cell populations of interest. The analysis is performed on multiple fine needle aspirates suspended in specific preservative solutions (generally provided by the laboratory running the test). The submitted sample must be fresh and ideally should be analysed within 24 hours of the time of collection (Guzera et al, 2014).

The presence of a monomorphic population of lymphoid cells, all expressing the same phenotype, is generally supportive of lymphoma, whereas a mixed lymphoid population expressing different phenotypes (likely a mixture of B and T cells) is more commonly associated with inflammatory and/or reactive processes. The prognostic significance of the immunophenotype in feline lymphoma is still unclear (Patterson-Kane et al, 2004).

PCR for antigen receptor rearrangement

PARR is a clonality assay based on the identification and amplification of the T-cell receptor and immunoglobulin genes, in T and B lymphoid cells respectively. The demonstration of a clonal population of either T or B cells generally supports a neoplastic origin. This technique is particularly useful in equivocal cases when both cytological and histopathological results are ambiguous. Published studies report variable sensitivity and specificity for this technique, which, ideally, should be applied not as a definite, but an adjunctive, tool for diagnosis and characterisation of feline

lymphoma (Sato et al, 2011).

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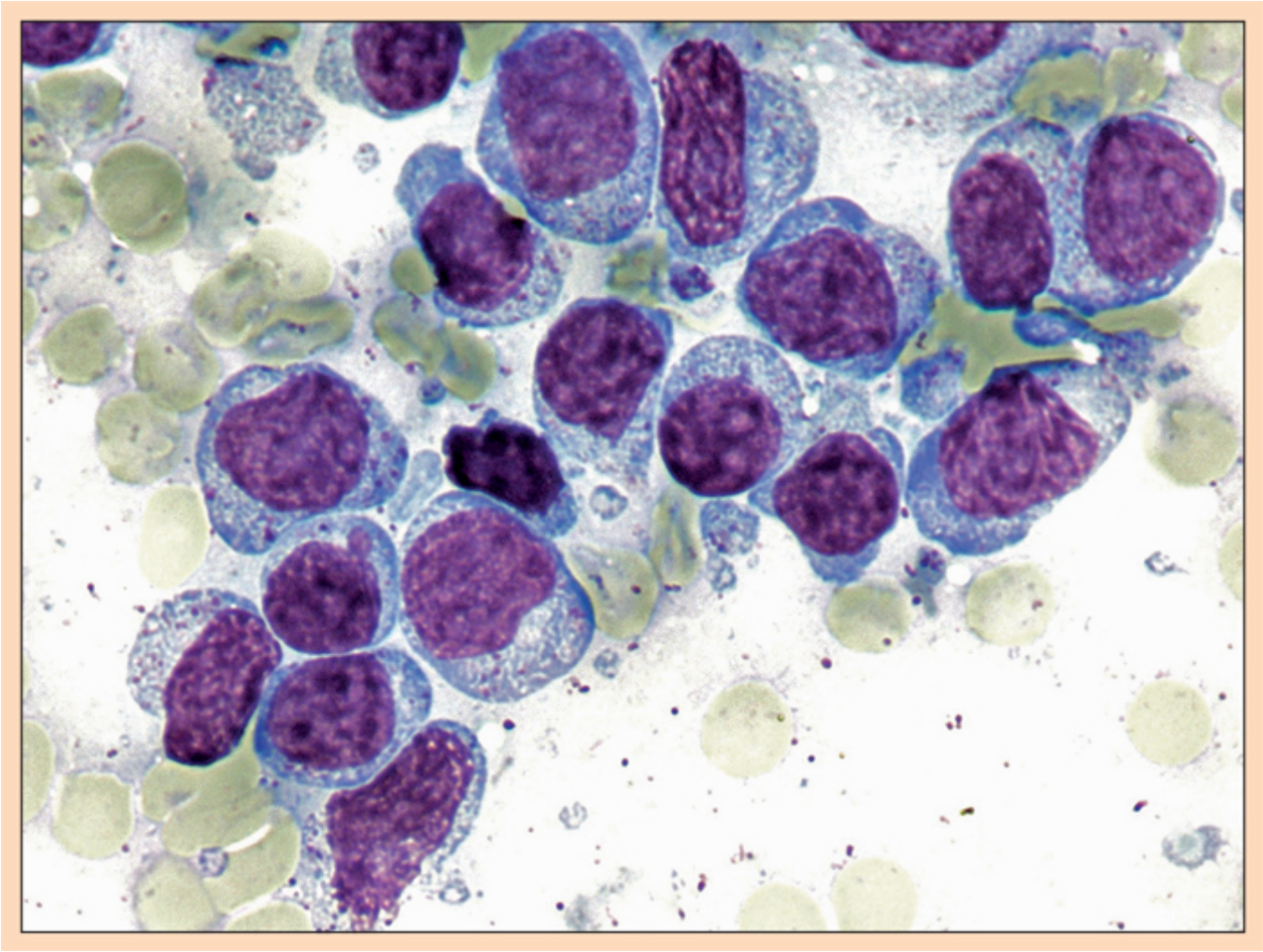

Figure 1. Fine needle aspirate from a cat with intestinal large granular lymphocytes lymphoma. Monomorphic population of large lymphoid cells containing azurophilic (purple) intracytoplasmic granules (Wright-Giemsa).

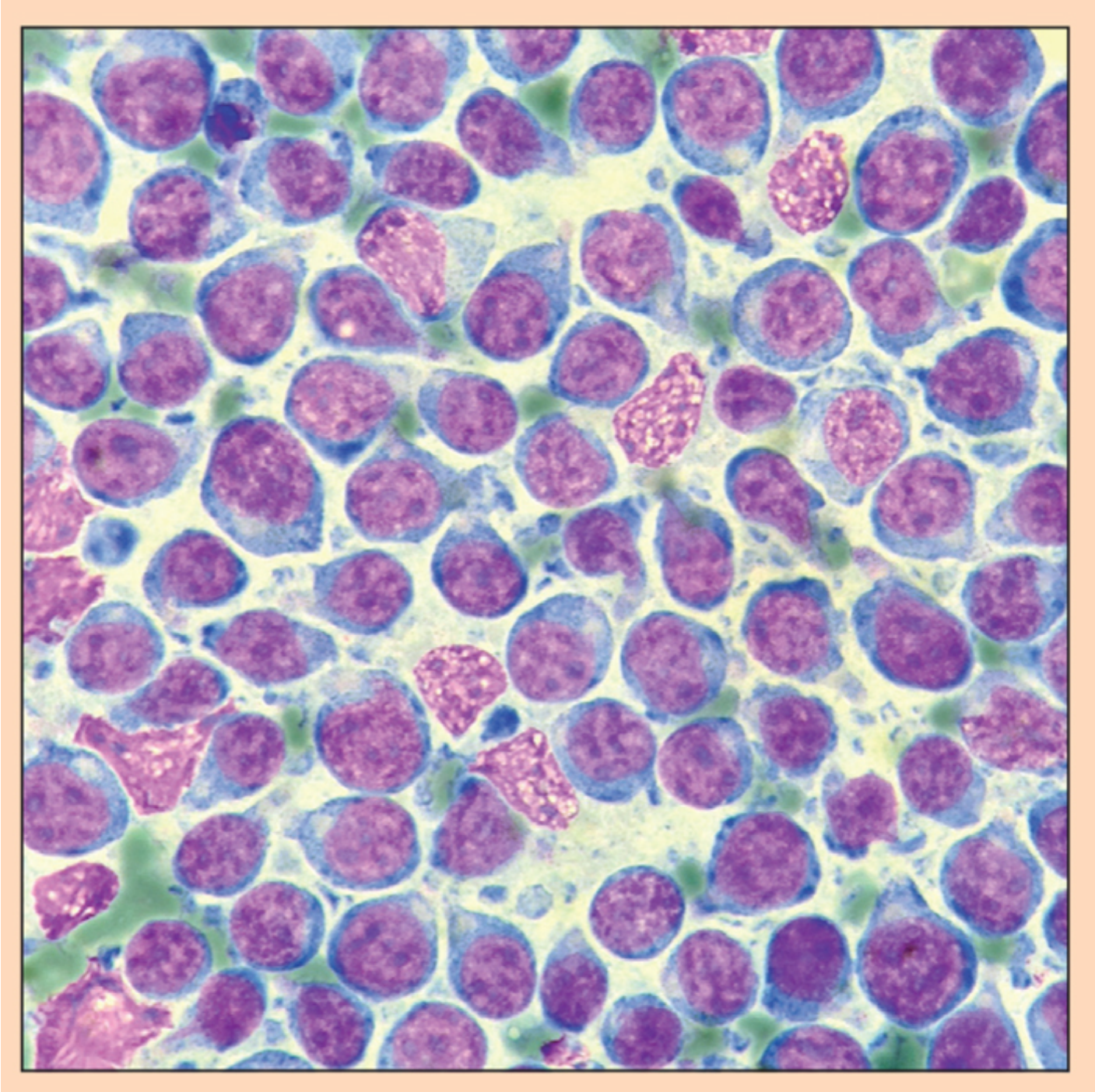

Figure 2. Fine needle aspirate from a cat with intestinal large cell lymphoma. Monomorphic population of large lymphoid cells characterised by moderate amounts of basophilic cytoplasm and a paracentral, round, occasionally indented nucleus with coarse granular chromatin and multiple

round nucleoli (Wright-Giemsa).

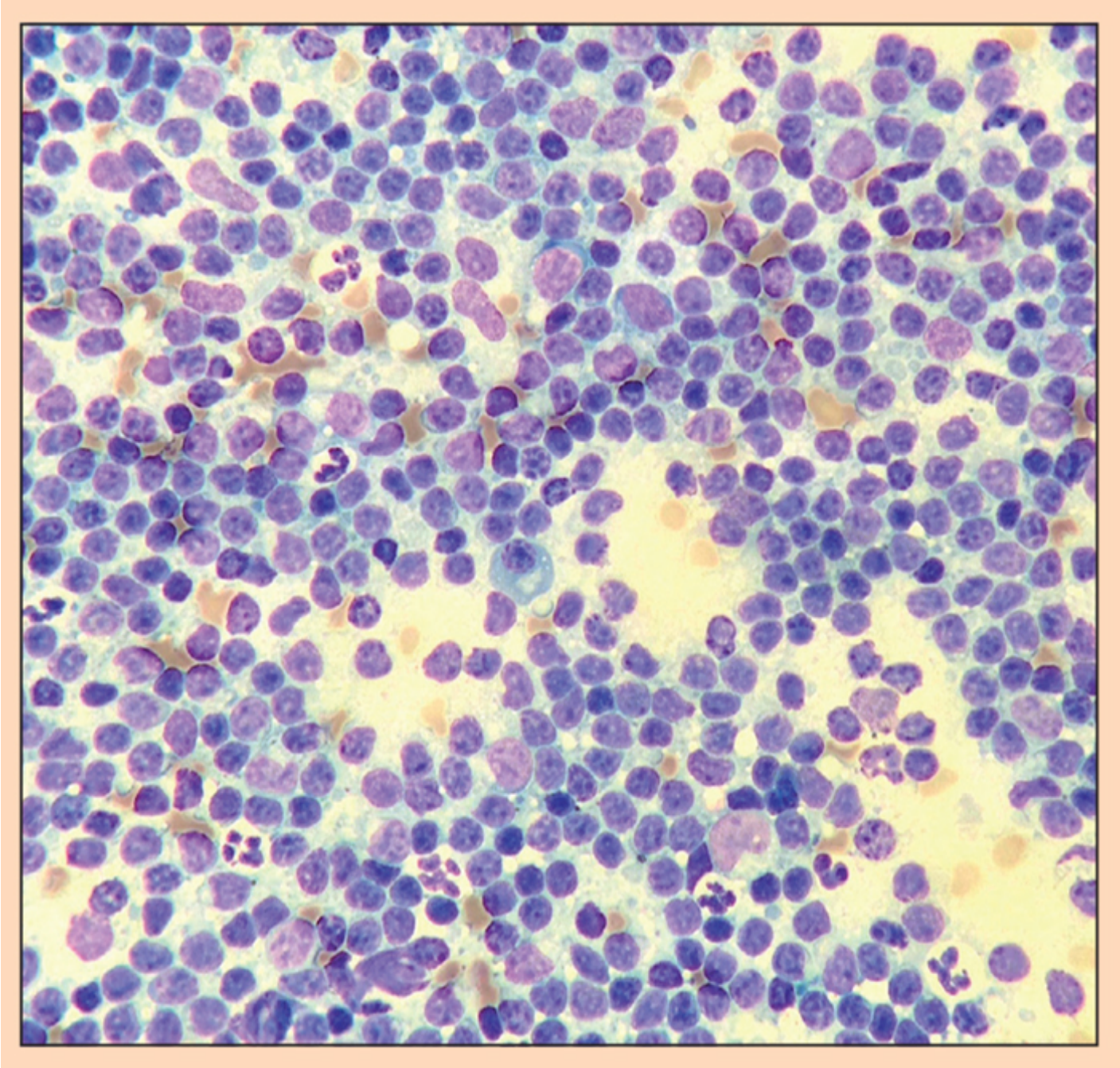

Figure 3. Fine needle aspirate from the submandibular lymph node of a cat with reactive lymphoid hyperplasia secondary to stomatitis of the oral cavity. Mixed population of lymphoid cells, mostly small lymphocytes, with a lower percentage of intermediate/large forms and occasional plasma cells (Wright-Giemsa).

Cell size	Small	One to 1.5 RBC
	Intermediate	Two to 2.5 RBC
	Large	More than three RBC
Mitotic activity	Low	Zero to one mitoses in five HPF 50×
	Moderate	Two to three mitoses in five HPF 50×
	High	More than three mitoses in five HPF 50×
RBC = red blood cells; HPF = high power field		

Table 1. Cytologic classification of lymphoma (Raskin, 2010)