RESEARCH ARTICLE
JIVA 18 (2) August 2020

Received: 29.04.2020
Accepted: 19.06.2020

A STUDY ON CYTOLOGICAL DIAGNOSIS OF CANINE LYMPHOMAS

Devi, S.S.[1*], Ajith, J.G.[2], Ashna, S.[3] and Sajitha, I.S.[4]

[1,3,4] Department of Veterinary Pathology,
College of Veterinary & Animal Sciences, Mannuthy
[2] Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Pookode
**Corresponding author: deviss@kvasu.ac.in*

ABSTRACT

One of the most important malignancies affecting dogs is the malignant lymphomas. Though histopathology, flow cytometry and immunohistochemistry are used as golden standard tools for confirmation of lymphomas, cytological evaluation of the fine needle aspirate from the lymph nodes still remains as the most basic method for primary diagnosis. Considering the minimal invasiveness, low cost and minimal time taken by this procedure for reaching the diagnosis, cytological examination is the most accepted method for preliminary diagnosis of lymphomas. Hence, the study was undertaken to characterize the different cytological features that help in differentiating neoplastic cells from lymphoid cells in various stages of development. Out of total twenty aspirates that were screened, fifteen were diagnosed as lymphomas. The present study helped in identifying many typical cytological characteristics that can be used for grading canine lymphomas.

Key words: Cytology, Malignancy, Grading, Lymphomas

INTRODUCTION

Lymphoma is a common type of neoplasia in canines with an estimated annual incidence rate of 13-33 cases per 1,00,000 dogs (Regan *et al*., 2012) and often morphological features are sufficient for a primary diagnosis. This is because a vast majority of canine lymphomas belong to four or five categories characterized by a typical cytological picture. Hence in veterinary medicine, excisional biopsy of the lymph node and histopathology are recommended only when the cytological pictures fail to give a conclusive result. Moreover, cytology is a cheap, easy to perform, safe and reliable method of lymphoma diagnosis when compared to histopathological examination (Amores - Fuster *et al*., 2015). A classification system

referred to as updated Kiel Classification (uKC) is commonly adopted for subtyping of canine lymphomas. In this system, general cytological diagnosis based on the percentage of immature cells in the smears and characters such as cell and nucleus sizes, distribution and basophilia of cytoplasm, shape of the nucleus, chromatin structure, presence and distribution of nucleoli and mitotic figures are considered. The system also associates the morphology of neoplastic cells with their immunophenotypes which is very much helpful in the accurate diagnosis of canine lymphomas. Hence in the present study, an attempt has been made to identify the various cytological characteristics that are helpful in grading canine lymphomas along with identification of the immunophenotype of the cells involved.

MATERIALS AND METHODS

A total of twenty cases that were suspected of lymphoma formed the study material. The criteria for selection of cases under this study were;

1. A marked lymphocytosis and presence of immature as well as atypical lymphoid cells in blood smear
2. Generalized lymphadenopathy with associated paraneoplastic syndromes like general malaise, weight loss and emaciation, fever etc.
3. Presence of perceptible cutaneous masses along with lymphocytosis and lymphadenopathy
4. Presence of lymphoid series of cells in the fluid collected by thoracocentesis or abdomeno-centesis

In the above cases, fine needle aspirate of the superficial lymph nodes preferably popliteal or prescapular nodes were collected using a needle of 23-25 gauge. The collected cellular material was then made into smears on slides and stained with Leishman-Giemsa and examined under oil immersion objective of the microscope. Cytomorphologic features and degree of anaplastic changes were studied in each case.

RESULTS AND DISCUSSION

Out of the twenty cases examined for cytology, five were excluded from the study as they represented inflammatory and reactive or hyperplastic lymph nodes. Inflammatory nodes were characterized by numerous neutrophils, occasional macrophages and a few RBCs. A reactive lymph node was confirmed when the proportion of immature cells per high power

field fell below 20 per cent of the total lymphocytes counted. The remaining fifteen smears revealed lymphomas of different types and varying grades. The different features that helped in classification and grading were

a. Nuclear size

The first criterion adopted for classification was based on the size of the cells/ nuclei. If the nuclear size was larger than the combined diameter of two red blood cells, such cells were regarded as large cells and if the size was less, they were considered as small cells. The percentage of small and large lymphoid cells was used as the criteria for describing different types of lymphoma. Depending on the proportion of small cells in the total population of neoplastic cells, lymphomas were classified as large cell, mixed cell and small cell lymphomas. If the total cell population comprised of only less than 50 per cent of small cells, it was classified as large cell lymphoma (Fig. 1). Similarly if the total cell population was about 50-70 per cent of small cells, it was identified as mixed cell lymphoma (Fig. 2) and if the total per cent of small cells was above 70, it was described as small cell lymphoma. Out of the fifteen lymphoma cases, seven were large cell lymphomas, six were of mixed type and two were small cell lymphomas.

b. Mitotic activity

Grading of the cases was carried out using mitotic activity as an indicator. Lymphomas with majority of small cells and low mitotic activity were classified as low grade lymphomas while lymphomas with majority of large and medium sized cells with high mitotic indices were regarded as high grade lymphomas. Mitotic index was estimated by scanning several fields and counting mitotic figures (Fig. 3). In the study, ten cases were identified as high grade lymphomas and remaining five were of low grade. The result was in agreement with that of earlier workers where they observed that majority of lymphomas in dogs were of high grade (Ponce *et al.*, 2010; Sapierzyński *et al.*, 2012).

c. Cellular morphology

The cellular population in different cases of lymphoma consisted of variable morphology. In majority of the large and mixed cell lymphoma cases, the cells were monomorphic and resembled immature blasts and they were regarded as lymphoblastic lymphoma (Fig. 1). While in two of the cases, the cells resembled monocytes and they were classified as monocytoid lymphoma (Fig. 4). Morphology of cellular population was also used as an indicator of immunophenotype. Fournel-Fleury *et al.* (2002), identified that

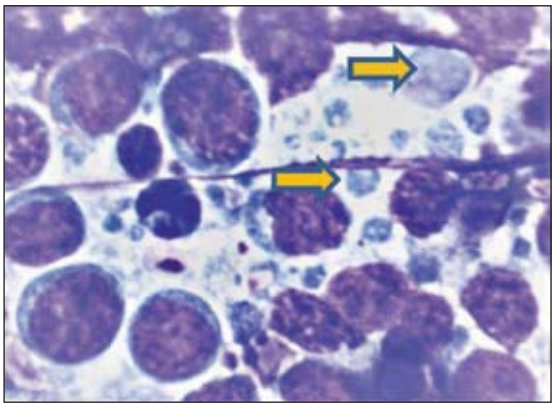

Fig. 1
Large cell lymphoma consisting of immature lymphoblasts and scattered small round or irregular shaped LGBs (Arrows)

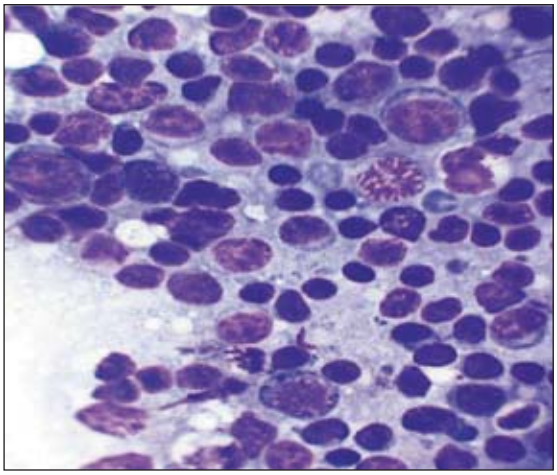

Fig. 2.
Mixed cell lymphoma

plasmacytoid morphology of neoplastic cells suggested a T-cell lymphoma. In the present study, none of the cases showed a plasmacytoid morphology of cells.

d. Presence of Lympho Glandular Bodies (LGBs) and Tingible Body Macrophages (TBMs)

Other cytomorphological features observed in the study included Lympho Glandular Bodies (LGBs) and Tingible

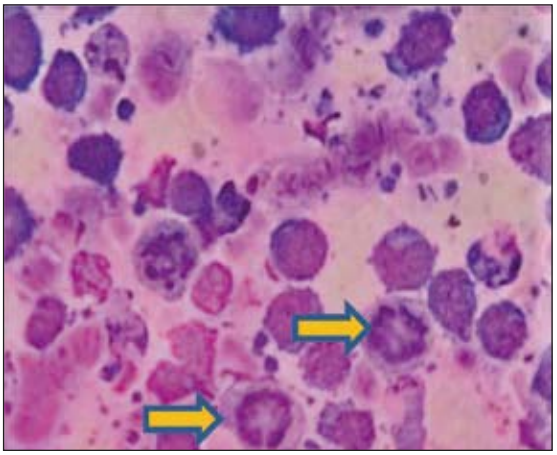

Fig. 3
Mitotic figures (Arrows)

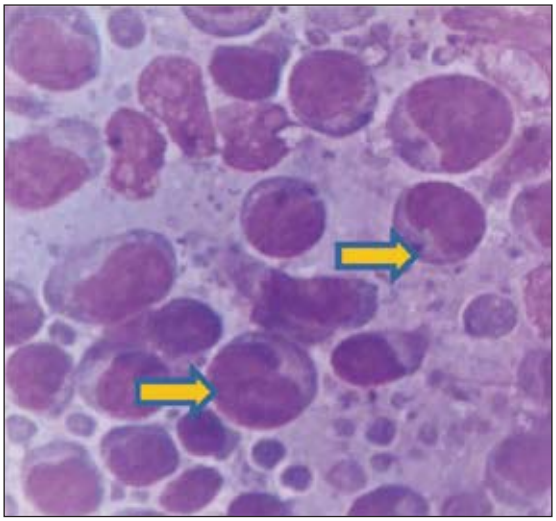

Fig. 4
Monocytoid cells (Arrows)

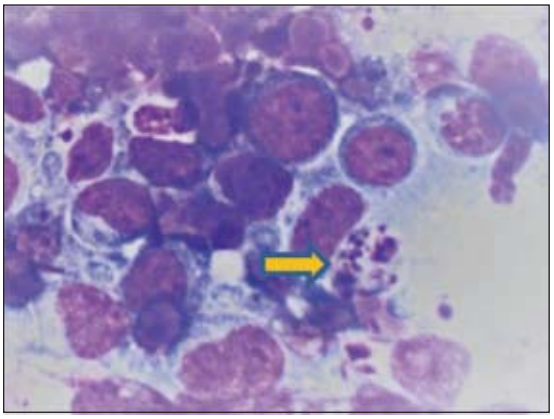

Fig. 5.
TBM (Arrow)

Body Macrophages (TBMs). LGBs appeared as small basophilic round or irregular remnants of cytoplasm with smooth borders and were seen scattered amongst intact lymphocytes (Fig. 1). TBMs were observed among neoplastic lymphoid cells and contained phagocytized apoptotic bodies and remnants (Fig. 5). Those cases showing significant numbers of LGBs and TBMs were regarded as high grade lymphomas. Also several studies have shown that LGBs are numerous in B cell lymphomas. Moreover the presence of LGBs helps in differentiating lymphomas from other malignant round cell tumours. TBMs are also regarded as hallmark of high grade B cell lymphomas which can never be significantly present in low grade B cell lymphomas or T cell lymphomas (Sapierzyński *et al*., 2016). In this study, LGBs and TBMs were appreciably noticed in two cases each, thus suggesting the involvement of neoplastic B cell population.

SUMMARY

Cytological evaluation of fine needle aspirate of the lymph node smears from the above fifteen cases suggested that large cell, lymphoblastic, high grade lymphomas are more in canines. Regarding immunophenotype, majority of the cases indicated B cell involvement with respect to their morphological features. Though updated Kiel classification system suggests a strong relationship between the morphology of neoplastic cells and their immunophenotype, disagreements have been reported by some of the workers. Hence flow cytometry or immunocytochemistry should be employed for confirmation of immune phenotypes. However, cytological evaluation of lymph node fine needle aspirates can be of much use in both diagnostic as well as prognostic points of view in canine lymphomas. Thus cytodiagnosis provides an almost accurate indication of grades and an overview of immunophenotype of neoplastic cells in canine lymphomas.

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