CYTOPATHOLOGICAL DIAGNOSTIC IN CANINE LYMPHOMA

Ozana Maria HRIȚCU, Sorin-Aurelian PAȘCA
USAMV Iași
Aleea Mihail Sadoveanu, Nr. 8, Iași
ozana.hritcu@uaiasi.ro

Abstract
Lymphomas are tumors that may be frequently diagnosed in dogs. The main suspicion for this neoplasic disease arises when lymphnodes become enlarged without the existence of any obvious antigenic stimulation. Fine needle aspiration from enlarged lymphnodes with the preparation of cytological slides allows for an early and relatively precise diagnostic in lymphoma cases. We used this technique, along with the May Grunwald Giemsa staining method to search for cytological and nuclear abnormalities that might indicate the evolution of a lymphoma in dogs that presented with enlarged lymphnodes. We found a constant mixture of medium sized and large lymphocytes, multinucleated cells, anisokaryosis, various nuclear shapes (multiple indentations), multiple visible nucleoli, variable cytoplasmic/nuclear ratios and cytoplasmatic chromatic properties (basophilia, light basophilia). Also, the mitotic index measured per 5 HPF fields (x400) was sometimes a strong indicative of malignancy. All of the cases diagnosed with lymphoma based on cytopathological examination were later confirmed through histopathological examination. This suggests that this simple and fast technique that is applicable also in small animal practices may be used to diagnose lymphomas in dogs with a relative high accuracy.
Keywords: *cytopathology, lymphoma, dog, cat*

Introduction

Lymphoma is a malignant tumor of the lymphatic tissue, that may manifest in various forms and degrees of malignity. It is a disease that usually may be diagnosed only after a histopathological examination of tissue samples from the affected organs (lymphnodes, liver, intestine, skin). However, in private practices the access to such diagnostic methods may be limited and the time aspect may be of great importance when considering tumor disemination, metastasis, organ failure or the surviral time of the patient (Grant, 2016).

Cytopathological diagnosis on the other hand may provide a compromise between the accuracy of the diagnosis, the complexity of the technique, the cost and time needed to reach a diagnosis and the level of invasiveness (Ressel, 2018).

The cytological features in lymphoma, as described by the literature, include an increased proportion of medium (nuclei 2-2,5 times the size of red blood cell) and large (nuclei 3 times the size of a red blood cell or larger), immature lymphocytes (above 20%), with large and lightly pigmented nuclei and a higher amount of lightly basophilic cytoplasm than the one normally seen in mature lymphocytes. Also, an increased rate of anisokaryosis, anisocytosis, indented nuclei, visible, multiple or indented nucleoli constitute strong indicatives for lymphoma. The mitotic rate is calculated in cytopathology for 5 random high power fields (x400) and expressed as follows: low when zero or one mitotic figure has been noticed, moderate for 2-3 mitosis and high is the count is above 3 (Ressel, 2018; Meuten, 2017; Raskin, 2016).

Material and methods

Fine needle aspiration from enlarged lymphnodes with the preparation of cytological slides allows for an early and relatively precise diagnostic in lymphoma cases. We used this technique, along with the May Grunwald Giemsa staining method to search for cytological and nuclear abnormalities that might indicate the evolution of a lymphoma in dogs that presented with enlarged lymphnodes (Raskin, 2016, Dunn, 2014).

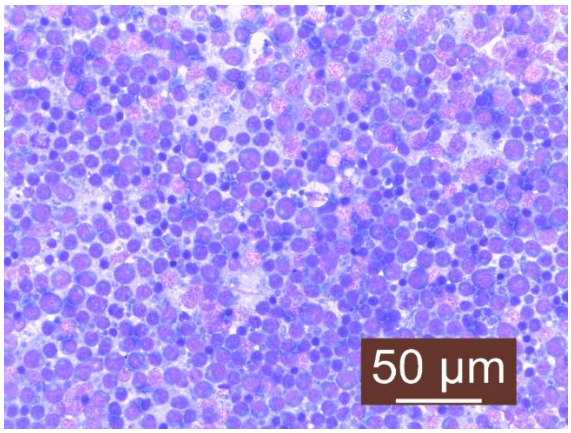
The lymphnodes were punctured using a 23 G needle attached to a seringe. Aspiration was done whilst moving the needle with a fan-like motion, Negative presure was released before

retracting the needle and a new seringe filled with air was reattached to it. The content of the needle was ejected on a slide and lightly compressed between that and another glass slide. MGG staining method followed the standard protocol: 3 min for May Grunwald, 3 min distilled water and 25 min for Giemsa solution (Dunn, 2014).

Examination of the slides was done using a Leica DM 750 optical microscope.

Results and discussion

In all the examined slides we found a constant mixture of medium sized and large lymphocytes (Fig. 1, 2), indicating a continuous process of formation of young, immature lymphocytes with abnormal characteristics. Among these we noticed anisokaryosis, various chromatin patterns (Fig. 3), various nuclear shapes (sometimes with multiple indentations), multiple visible nucleoli (sometimes indentated), variable cytoplasmic/nuclear ratios and cytoplasmatic chromatic properties (basophilia, light basophilia).



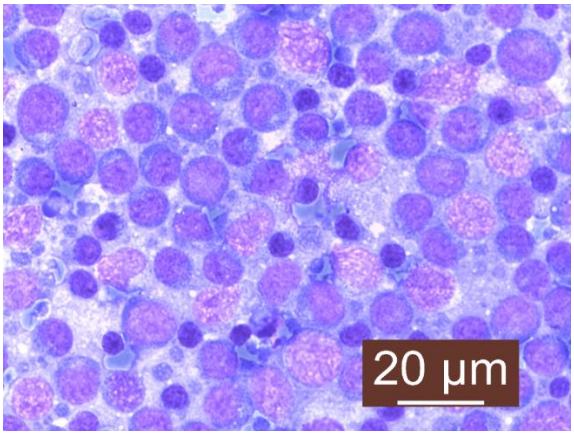


Fig. 1, 2 A mixture of small, medium and large lymphocytes may se seen, with both nuclei and cytoplasm dysplaing various aspects. Lymphoma. FNA from lymphnode. Dog. Masson trichrome stain

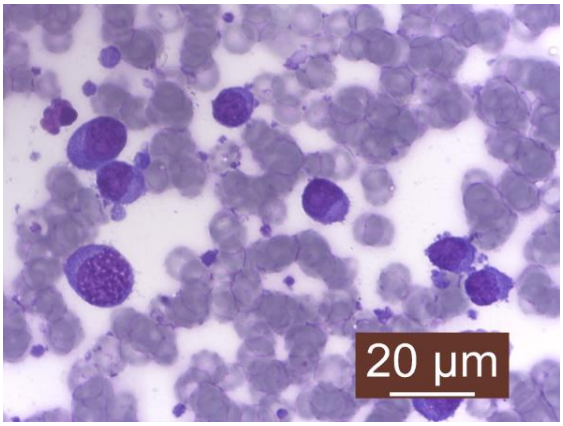


Fig. 3 Stappled cromatin and anisokaryosis. Lymphoma. FNA from lymphnode. Dog. Masson trichrome stain

Sometimes, abnormal cells with multiple nuclei could be observed, the nuclei being of the same or of different sizes (Fig. 4, 5). The cromatin pattern varied between the nuclei of different cells or the ones of the same cell, being either granular, stappled or condensed.

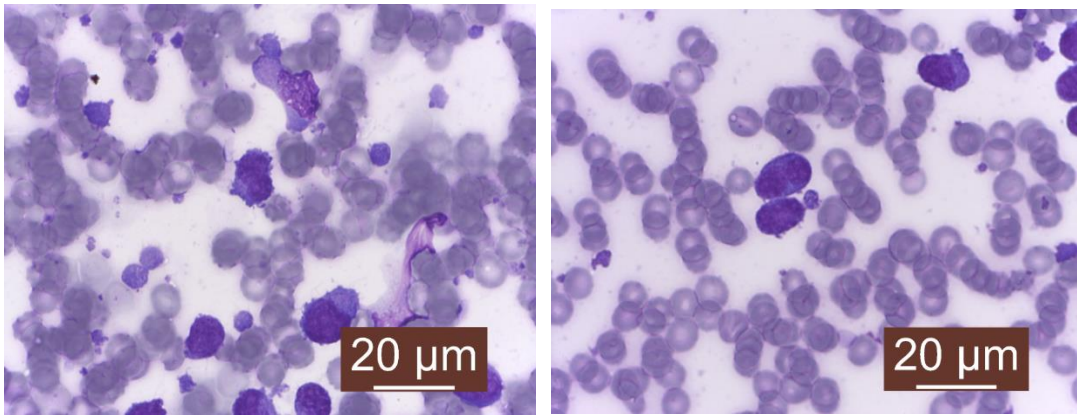
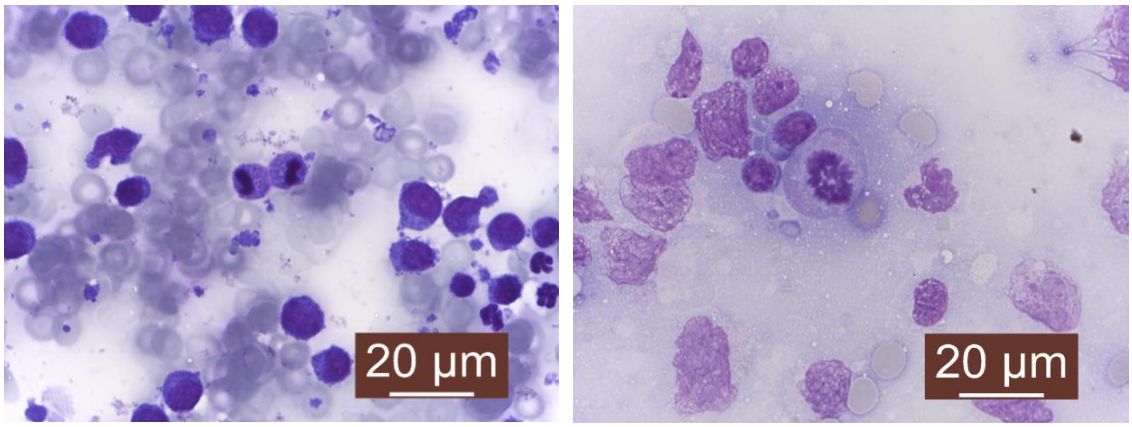


Fig. 4, 5 Multinucleated lymphocytes are also a malignancy criteria. Lymphoma. FNA from lymphnode. Dog. Masson trichrome stain

Also, the mitotic index measured per 5 HPF fields (x400) was sometimes a strong indicative of malignancy. We were able to capture different phases of the mitosis and even found high power fields with 5 mitotic figures, indicating an active proliferative process (Fig. 6-9).



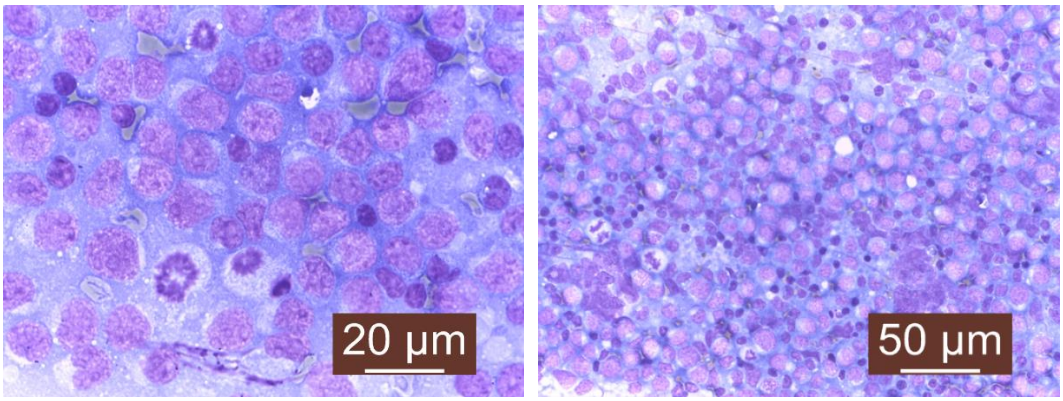


Fig. 6-9 Mitotic figure, both normal and abnormal, sometimes found in high numbers in a single high power field. Lymphoma. FNA from lymphnode. Dog. Masson trichrome stain

All the cases diagnosed with lymphoma based on cytopathological examination were later confirmed through histopathological examination. These results suggest that this simple, unexpensive and fast technique that is applicable also in small animal practices may be used to diagnose lymphomas in dogs.

The widespread of this procedure could help in establishing an earlier diagnostic in lymphomas in dogs, in formulating a more acurate prognostic and in initiating a treatment plan that may help prolongue the life of the patient or prevent metastasis.

Conclusion

Cytopathological diagnosis is a viable option in establishing a diagnosis with relatively high accuracy in canine lymphoma, in lack of or while waiting for the posibility of perfoming techniques and exams that can provide for a more accurate diagnosis.

References

1. Meuten J. Donald (2017) – Tumors in domestic animals. Fifth edition. Wiley Blackwell, Raleigh, USA;
2. Ressel Lorenzo (2018) – Normal cell morphology in Canine and Feline Cytology. An Identification Guide. Wiley Blackwell, Liverpool, UK;
3. Raskin E. Rose, Meyer J. Denny (2016) – Canine and feline cytology. A color atlas and interpretation guide. Elsevier, St. Louis, USA;
4. Dunn John (2014) – Manual of diagnostic cytology of the dog and cat. Wiley Blackwell, Chichester, UK
5. Grant M. Maxie (2016) – Jubb, Kennedy and Palmer's Pathology of domestic animals. Vol. III. Sixth edition. Elsevier. St. Louis, USA.