Cytological diagnosis of multicentric lymphoma stage IVb characterized by a uniform population of lymphoblasts

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Abstract. A 10-year-old female Doberman weighing 31.5 kg was presented to the Prof. Soeparwi Veterinary Teaching Hospital with complaints of ocular discharge and lethargy. Clinical examination revealed signs of dehydration, including a capillary refill time exceeding 2 seconds and pale mucous membranes. Hematological analysis indicated persistent non-regenerative anemia, leukopenia, thrombocytopenia, and elevated levels of SGOT, amylase, and creatine kinase. Subsequent examination revealed abdominal distension and peripheral lymphadenopathy. Abdominal ultrasonography and radiography confirmed splenomegaly and multiple enlarged lymph nodes. Based on these findings, an ultrasound-guided fine-needle aspiration (FNA) was performed on the axillary, popliteal, and abdominal lymph nodes, followed by cytological evaluation using Diff-Quick staining. Cytological examination revealed a monomorphic population of lymphoblasts with a diffuse chromatin pattern, which suggest a diagnosis of lymphoma. Considering the clinical presentation and diagnostic laboratory findings, the case was diagnosed as multicentric lymphoma, stage IVb. The prognosis was considered infaust. Palliative treatment with prednisone (2 mg/kg BW) was initiated; however, the patient's condition progressively deteriorated, and euthanasia was decided on day 14 post-diagnosis. This case emphasizes the importance of early diagnosis, precise staging, and comprehensive client communication in the effective management of canine lymphoma. Chemotherapeutic intervention remains the most efficacious therapeutic strategy and should be strongly considered as the primary treatment modality for lymphoma.

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1 Introduction

Lymphoma accounts for 24% of all documented canine neoplasms and 85% of hematological malignancies, while multicentric lymphoma corresponds to 84% of all canine lymphomas [1]. It arises from the clonal proliferation of lymphoid cells at various stages of differentiation, most commonly of B-cell or T-cell origin [2]. The disease exhibits substantial clinical and biological heterogeneity, ranging from indolent to highly aggressive subtypes, with middle-aged to older dogs being most frequently affected [3]. Canine lymphoma are classified according to their anatomical location, histologic characteristics, and immunophenotype [4, 5].

The multicentric form, characterized by generalized lymphadenopathy, is the most common clinical presentation of canine lymphoma [2]. However, extranodal forms that affect the skin, gastrointestinal tract, or mediastinum often complicate diagnosis and management [6]. Multicentric lymphoma accounts for approximately 84% of all canine lymphomas [2], with reported prevalence ranging from 53% to 83% [4,5]. It is typically characterized by superficial, non-painful lymphadenopathy [1]. A definitive diagnosis is established through a comprehensive approach incorporating clinical assessment, cytologic examination, histopathologic evaluation, and immunophenotyping. More recently, molecular assays such as PCR for antigen receptor rearrangements (PARR) have become invaluable tools for confirming clonality and differentiating neoplastic lymphoid proliferations from reactive hyperplasia, which can be histologically similar [7-8].

Multi-agent chemotherapy, particularly the CHOP-based regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone), remains the standard treatment for canine lymphoma [1]. This protocol typically achieves high remission rates and provides a median survival time of 9 to 12 months in most affected dogs [2, 6]. Nevertheless, relapse and multidrug resistance are frequent, emphasizing the need for novel prognostic markers and targeted molecular interventions. Dysregulation of signaling pathways such as PI3K/AKT, JAK/STAT, and NF-κB has been implicated in the oncogenic process and therapeutic resistance in both canine and human lymphomas [9].

Early diagnosis is a critical factor influencing treatment response and overall outcome in canine lymphoma [1, 3]. Detecting the disease before significant progression allows for timely therapeutic intervention, which can greatly improve remission rates and survival time. Cytologic examination, particularly fine-needle aspiration (FNA), serves as a valuable first-line diagnostic tool because it is rapid, minimally invasive, and capable of providing essential information about the nature of lymphoid proliferation. In this case report, we present a canine lymphoma case emphasizing the cytological findings as the initial diagnostic approach.

2 Case presentation

A 10-year-old, 31.5 kg female Doberman was admitted to the Prof. Soeparwi Veterinary Teaching Hospital. The owner reported ocular discharge and lethargy. The dog exhibited polydipsia and polyuria. According to the owner's information, this condition had been occurring for approximately one week prior to examination. Physical examination showed capillary refill time more than 2 seconds and abdominal enlargement. Based on these findings, splenomegaly was suspected. On palpation, enlargement of the superficial lymph nodes was observed, particularly the submandibular, axillary, and popliteal nodes.

2.1 Hematological examination

The initial hematological examination revealed decreased packed cell volume (PCV), non-regenerative anemia, leukopenia, thrombocytopenia, and increased levels of serum glutamic oxaloacetic transaminase (SGOT), amylase, and creatine kinase. A subsequent hematological evaluation demonstrated persistent non-regenerative anemia, thrombocytopenia, and elevated serum glutamic pyruvic transaminase (SGPT) and amylase levels.

Table hematology examination

Parameter	Abbre-viation	Unit	Result	Normal range*	Interpretation
Red blood cell	RBC	10^6/µL	4.71	5.50 – 8.50	Low
Hemoglobin	HGB	g/dL	9.9	12.0 – 18.0	Low
Hematocrit	HCT	%	30.1	37.0 – 55.0	Low
MCV	MCV	fL	63.9	66.0 – 77.0	Low
MCH	MCH	pg	21.0	19.5 – 26.0	Normal
MCHC	MCHC	g/dL	33.0	32.0 – 36.0	Normal
White blood cell	WBC	10^3/µL	8.17	6.0 – 17.0	Normal
Neutrophils percentage	Neu %	%	85.2	60.0 – 77.0	High
Neutrophils number	Neu	10^3/µL	6.97	3.0 – 11.5	Normal
Basophils percentage	Bas %	%	0.0	0.0 – 1.0	Normal
Basophils number	Bas	10^3/µL	0.0	0.0 – 0.1	Normal
Eosinophils percentage	Eos %	%	0.5	2.0 – 10.0	Low
Eosinophils number	Eos	10^3/µL	0.04	1.0 – 1.25	Low
Lymphocytes percentage	Lym %	%	11.4	12.0 – 30.0	Low
Lymphocytes number	Lym	10^3/µL	0.93	1.0 – 4.80	Low
Monocytes percentage	Mon %	%	2.9	3.0 – 10.0	Low
Monocytes number	Mon	10^3/µL	0.23	0.18 – 1.35	Normal
Platelet count	PLT	10^3/µL	98	200 – 500	Low

Blood Chemistry

Parameter	Unit	Result	Normal range	Interpretation
Liver Function				
GPT/ALT	IU/L	116.0	5.0 – 125	Normal
GOT/AST	IU/L	81.0	0.0 – 50	High
Total Bilirubin	gr/dL	0.3	0.0 – 0.9	Normal
Protein plasma	gr/dL	7.1	4.9 – 8.2	Normal
Globulin	gr/dL	4.0	1.9 – 4.5	Normal
Albumin	gr/dL	3.1	2.3 – 4.0	Normal
Ratio Albumin-Globulin		0.79	0.75 – 1.9	Normal
Glukose	mg/dL	88.0	74.0 – 143.0	Normal
Triglyceride	mg/dL	93.5	0.0 – 98.3	Normal
Kidney Function				

BUN	mg/dL	12.1	7.0 – 27.0	Normal
Creatinin	mg/dL	0.7	0.3 – 1.8	Normal
Ratio BUN/Creatinin		69	16.0 – 218	Normal
Electrolite				
Calcium	mg/dL	9.7	7.9 – 12.0	Normal
Phosphate Anorganic	mmol/L	1.9	0.8 – 2.2	Normal
Other				
Amylase	IU/L			High
Creatinin Kinase	IU/L			High

2.2 Ultrasonography

Abdominal ultrasonography revealed splenomegaly and generalized lymphadenopathy (Fig. 1). In addition to superficial lymph nodes that were palpable, such as the submandibular, axillary, and popliteal lymph nodes, enlargement was also detected in the abdominal lymph nodes.

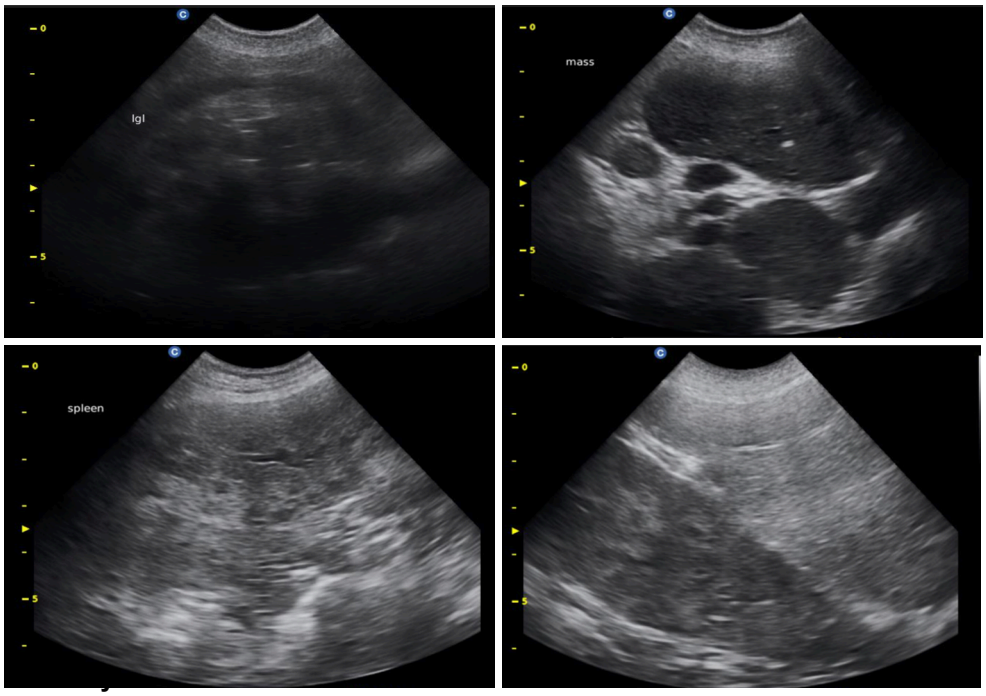

Fig. 1. Enlargement of lymph nodes was observed in multiple regions (A and B); The spleen appeared enlarged with a heterogeneous parenchymal texture resembling a honeycomb pattern (C); spleen echogenicity was increased, appearing more hyperechoic compared to the hepatic parenchyma (D).

2.3 Radiography

No evidence of pulmonary metastasis was detected on thoracic radiographs (Figure 2A). The radiographic findings indicated splenomegaly located in the ventral region of the abdomen (Figure 2B). The increased splenic size and displacement of adjacent intestinal loops further supported involvement of the spleen as part of systemic lymphoid neoplasia.

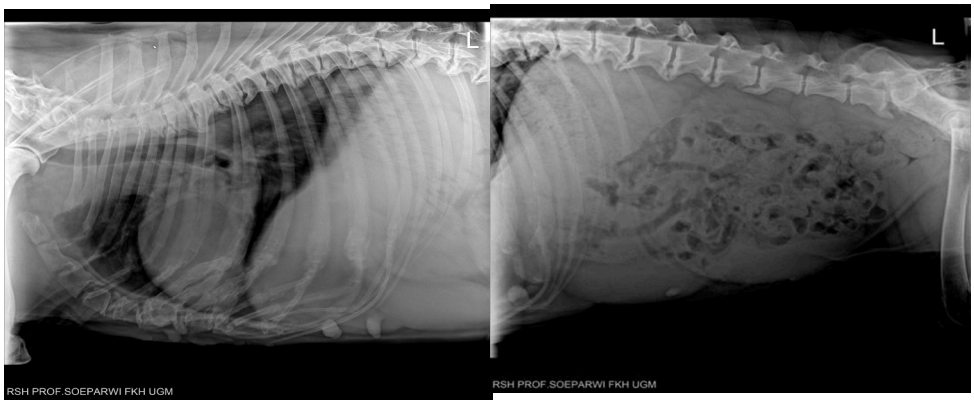

Fig. 2. Thoracic radiographs were obtained in left lateral projection. The cardiac and pulmonary silhouettes appeared within normal limits, and no nodular or interstitial patterns suggestive of metastatic lesions were observed (A); Abdominal radiographs revealed a marked enlargement of the spleen extending into the ventral abdomen (B).

2.4 Cytology

Cytological examination of the lymph node revealed a predominant population of large lymphoblasts (immature lymphocytes), accounting for more than 50% of the lymphoid cell population (Fig. 3).

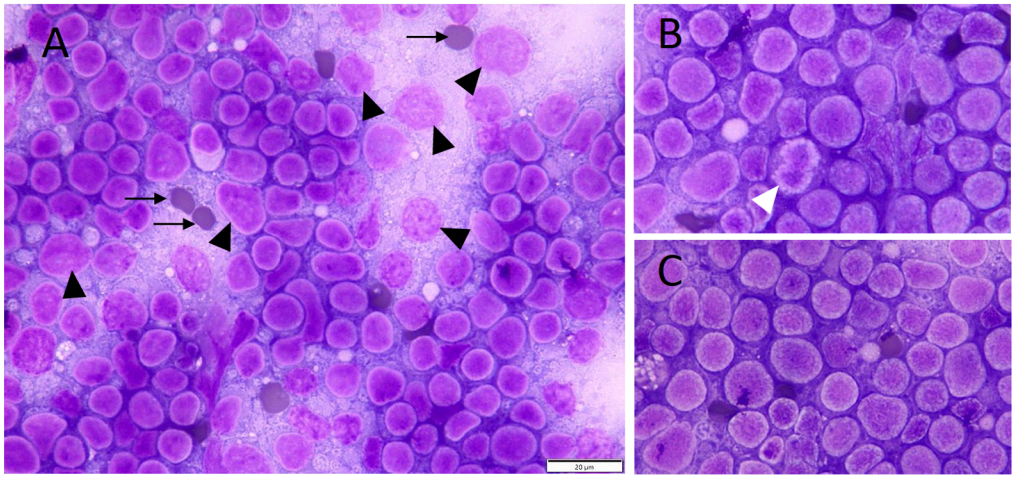

Fig. 3. The lymphoblasts (indicated by black arrowheads) appeared larger than erythrocytes (black arrows), with finely granular chromatin, a relatively low nucleus-to-cytoplasm (N:C) ratio, and basophilic cytoplasm (Figure A). The lymphocytes exhibited enlarged nuclei measuring approximately 1.5–2 times the

diameter of red blood cells. Several cells showed evidence of mitotic division (white arrowheads) and consistently displayed finely dispersed chromatin without visible nucleoli (Figures B and C). Diff-Quik stain, 1000× magnification

2.5 Diagnosis and outcome

Based on the clinical findings, diagnostic imaging, and cytological evaluation, a diagnosis of multicentric lymphoma, stage IVb, was established. The staging was determined according to the World Health Organization (WHO) classification system, indicating hepatic and splenic involvement (stage IV) accompanied by systemic clinical signs (substage b). The overall prognosis was considered poor. Although chemotherapy following the standard CHOP protocol was recommended, the owner declined this treatment option. Consequently, palliative therapy with prednisone (2 mg/kg body weight) was initiated. During the initial three days of treatment, the patient exhibited transient improvement, characterized by a return of appetite and increased activity. However, by the fourth day, the condition deteriorated, and euthanasia was elected 14 days after the initial diagnosis.

3 Discussion

The clinical, hematological, and imaging findings in this case are consistent with advanced multicentric lymphoma, a highly aggressive hematopoietic malignancy in dogs [2]. The patient presented with generalized lymphadenopathy, splenomegaly, and systemic clinical signs such as lethargy and prolonged capillary refill time, which collectively suggest disseminated disease. These manifestations reflect extensive neoplastic infiltration of lymphoid and parenchymal organs, particularly the spleen and liver, as evidenced by ultrasonographic findings showing a “honeycomb” appearance and diffuse hyperechogenicity of the splenic parenchyma. Such alterations are typical of lymphoid neoplastic infiltration resulting in disruption of normal splenic architecture.

The hematological findings in this case demonstrated persistent non-regenerative anemia, leukopenia, and thrombocytopenia, which collectively suggest bone marrow involvement, either through direct neoplastic infiltration or cytokine-mediated suppression of hematopoiesis. Anemia is a recognized paraneoplastic manifestation of lymphoma and is strongly associated with an unfavorable prognosis, with reported prevalence rates ranging from 30% to 77% [10]. Anemia in canine lymphoma is frequently associated with myelophthisis, while leukopenia and thrombocytopenia may result from impaired marrow function. These abnormalities indicate advanced disease progression and are often correlated with poor prognosis. In addition, the concurrent elevations of SGOT, amylase, and creatine kinase further support systemic involvement. Overall, these hematological alterations provide critical diagnostic and prognostic information, highlighting the significance of complete blood evaluation in assessing disease severity and guiding clinical management of canine lymphoma.

Cytological examination through FNA played a crucial role in establishing the diagnosis in this case. The identification of a homogeneous population of large lymphoblasts with fine chromatin and basophilic cytoplasm strongly indicated a high-grade lymphoma [10, 11]. Typically, neoplastic cells in lymphoma are large lymphoid cells, measuring more than twice the diameter of an erythrocyte or exceeding the size of a neutrophil [1]. These cells often exhibit prominent nucleoli and basophilic cytoplasm, or alternatively, possess finely dispersed chromatin with indistinct nucleoli. Cytology offers several advantages as a first-line diagnostic method in canine lymphoma: it is minimally invasive, rapid, cost-effective, and provides immediate diagnostic insight, which is essential for timely therapeutic decisions [6]. Moreover, in clinical settings where histopathology or

immunophenotyping may not be readily available, cytology can serve as a reliable preliminary diagnostic tool for differentiating neoplastic from reactive lymphoid hyperplasia. However, cytology also has certain pitfalls. It may not reliably distinguish between different lymphoma subtypes or identify the immunophenotype (B-cell vs T-cell origin) without adjunctive techniques such as flow cytometry or immunocytochemistry [10]. In addition, cytologic interpretation can be challenging in cases with mixed cell populations or necrotic samples, potentially leading to diagnostic ambiguity. Despite these limitations, in this case, cytology provided sufficient evidence to confirm a diagnosis of high-grade lymphoma, facilitating staging and prognostic assessment [1, 10].

According to Mantini et al. [11], cytology serves as a fundamental diagnostic tool for identifying lymphoma and can be employed as a screening method to predict tumor grade and phenotype. The diagnostic accuracy of cytology for canine lymphoma has consistently exceeded 80%, with an interobserver agreement coefficient of 0.55. The study also demonstrated that cytology is capable of effectively distinguishing between lymphoma and non-lymphoma lesions, with a high confidence level of 75.6%. Nevertheless, cytology alone has limitations in accurately determining tumor grade, immunophenotype, or WHO histopathological subtype. Therefore, complementary diagnostic techniques (such as flow cytometry, histopathology, and immunohistochemistry), are required to achieve precise characterization of lymphoma grade and phenotype [2, 7].

According to the World Health Organization (WHO) clinical staging system, this case was classified as stage IVb, indicating hepatic and splenic involvement (stage IV) accompanied by systemic clinical signs such as lethargy and anorexia (substage b). The ultrasonographic detection of splenic enlargement with diffuse parenchymal alteration, combined with elevated hepatic enzymes, justified the classification of visceral organ infiltration. Determining the WHO lymphoma subtype is important, as variations in histopathological classification can influence the tumor's clinical behavior, prognostic outlook, and responsiveness to therapy. Stage IVb lymphoma is generally associated with a poor prognosis, as extensive organ involvement and systemic illness often correlate with reduced responsiveness to chemotherapy and shorter survival times [2-3]

Table 2. Clinical staging system for canine lymphoma by the World Health Organization (WHO)

Stage	Characteristic
I	Involvement limited to a single node or lymphoid tissue in a single organ
II	Involvement of many lymph nodes in a regional area (±tonsils)
III	Generalized lymph node involvement
IV	Liver and/or spleen involvement (±stage III)
V	Manifestation in the blood and involvement of bone marrow and/or other organ systems (±stage I–IV)
	Each stage is subclassified into
a	Without systemic signs
b	With systemic signs

The rapid clinical decline despite palliative therapy underscores the aggressive nature of advanced lymphoma and highlights the critical importance of early detection. Early-stage lymphoma often presents with subtle lymphadenopathy or mild hematologic changes, which may be overlooked without thorough diagnostic evaluation. The two most reliable prognostic factors are immunophenotype and WHO substage [1, 3]. Dogs classified with WHO substage b disease generally have a poorer prognosis than those with substage a,

while individuals in stages I and II exhibit a more favorable outcome compared to dogs in the later stages of the disease. Detecting lymphoma before the onset of systemic signs enables timely initiation of multi-agent chemotherapy, significantly improving remission rates and median survival times [2]. The reported survival time in untreated CL is 4–6 weeks after diagnosis [3].

4 Conclusion

In this case, delayed presentation allowed disease progression to an advanced stage where only supportive therapy was feasible, ultimately resulting in euthanasia within weeks of diagnosis.Therefore, this case illustrates the clinical value of integrating cytologic evaluation as part of routine diagnostic work-up in dogs with generalized lymphadenopathy. Early cytologic screening can guide prompt staging and therapeutic decisions, potentially improving clinical outcomes even in aggressive lymphoma subtypes.

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