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Clinicopathological study of diffuse large B cell lymphoma in a Labrador dog: A case report

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Abstract
A five-year-old male, Labrador retriever dog weighed 35kg was presented to the Madras Veterinary College Teaching Hospital with the history of swelling on face, neck, leg and abdominal region. Clinical examination revealed enlargement of all the peripheral lymph nodes. Ultrasonography of abdomen revealed enlargement of mesenteric lymph node and iliac lymph nodes. Splenic images showed reticular pattern or Swiss cheese like appearance. Anatomically, it was classified as multicentric lymphoma. Hematology was normal and in serum biochemistry, increased level of total and direct bilirubin indicative of hepatic insufficiency and corrected by giving heptatonic and fluid therapy for a week. Fine needle aspiration cytology (FNAC) showed presence of large lymphoblastic cells with multiple nucleoli and a few mott cells. FNAC sample in normal saline was analyzed using flowcytometry with CD21 and CD3 monoclonal antibodies for immunophenotyping which showed 69.35% of aspirated cells were tagged with CD21 marker which indicated B type lymphoma. It was in stage 'IV substage 'a' based on physical, clinical and ultrasound examination. CHOP protocol was given and partial remission was observed after one week of treatment. Animal was died due to hepatic insufficiency. Overall survival time of the dog was 20 days. Post mortem examination revealed all the lymph nodes were enlarged and heart was rounded appearance. Histopathological examination revealed effacement of nodal structure, capsular thinning and compressed peripheral sinus. Neoplastic cells were filled the medullary cord and compressed sinuses. Histologically it was classified as diffuse large B cell lymphoma. Metastasis was observed in liver, kidney, heart, lungs, brain and testis.

Keywords: Canine, lymphoma, multicentric, metastasis, Labrador, cytology, pathology, flowcytometry, immunophenotype

Introduction
Lymphoma is the frequently diagnosed malignancies in the dog between 7-24% of all tumors and 83-90% of hematological cancers which represents the most commonly managed neoplasia in Veterinary Medical Oncology (Zandvliet, 2016) [1]. Although only a small proportion of dogs with lymphoma is truly cured, because of earlier diagnosis. Genetically, Labradors were not predisposed to lymphoma, but tended to develop lymphoma (Comazzi *et al*., 2018) [2]. Lymphoma was more common in middle to old aged group of dogs when compared to young aged group (Dorn *et al*., 1967) [3]. Diffuse large B-cell lymphoma (DLBCL) is the commonest lymphoma in both humans and dogs. Due to spontaneously high incidence, complex genetic interplay, aggressive clinical course, elevated frequency and the presence of an intact immune system, dogs with lymphoma are considered as an ideal comparative model for drug development for human lymphomas (Aresu *et al*., 2016) [4].

Diagnosis
In the present case, diffuse large B cell lymphoma was reported in a five year old male intact Labrador retriever weighed 35kg. It was brought to the Madras Veterinary College Teaching Hospital with the history of swelling on the face, neck, leg and abdominal region. The characteristic clinical sign of canine multicentric lymphoma was "non-painful enlargement of lymph nodes" associated with absence of "systemic signs of illness" as described earlier by Grow 2015 [5] was also observed in the present case. General clinical examination of all peripheral palpable lymph nodes namely submandibular, pre-scapular, axillary, inguinal and popliteal were examined and measured in all the three dimensions, namely cranial – caudal (CC), dorsal – ventral (DV) and medial - lateral (MV) using vernier caliper (Fig. 1) and they were severely enlarged as described by Aresu *et al*., 2015 [6] (Table 1).

Hematological values were within normal range. Elevation of total and direct bilirubin in biochemical examination, which was indicative of hepatic insufficiency and it was corrected by giving heptatonic and fluid therapy for a week.

Involvement of abdominal and thoracic lymph nodes was assessed by other imaging modalities such as ultrasonography and radiography. Abdominal ultrasonography revealed distended gall bladder, enlarged prostate, dilated intestinal loops and borders of the liver were rounded indicating hepatomegaly. Enlargement of mesenteric and iliac lymph nodes was observed as hypoechoic structures. Spleen images showed cattle reticulum like or swiss cheese like appearance. The length and width of all the lymph nodes were measured in ultrasound. The length and width of the inguinal lymph node was 30.64mm and 18.36mm respectively (Fig. 2). The ratio of length and width is around 1.66. Since the ratio is less than 2, it clearly indicates that it is a malignant form of canine lymphoma (Steinkamp *et al*. 1995) [7]. Similarly, the length and breadth of all lymph nodes were measured and the ratio was less than 2. The length, width and thickness of prescapular, submandibular, axillary, inguinal and popliteal lymph nodes were measured before and after one week of first dose of chemotherapy using vernier caliper (Fig. 1) and the values were mentioned in Table 1 (Childress *et al*., 2014) [8]. Based on the lesions in spleen and generalized lymphadenopathy including mesenteric and iliac lymph nodes, without clinical signs, the condition was graded as Stage 'IV' substage 'a', as per the WHO system of classification (Owen 1980) [9]. Anatomically, the present case was classified as multicentric form. (Ettinger, 2003) [10]

Fine needle aspiration cytology (FNAC) was performed which revealed presence of lymphoblastic cells which were two times larger than the size of erythrocytes. Neoplastic cells were medium to large sized cells, pleomorphic with multiple nucleoli which indicative of centroblastic type (Fig. 3) Mott cells with small dense basophilic nuclei and abundant eosinophilic cytoplasm composed of variably distinct eosinophilic globules called Russell bodies (Fig. 4) were noticed which gives plasmacytoid appearance (Stacy *et al*., 2009) [11]. Few mitotic figures were observed which was low grade type (Sözmen *et al*., 2005) [12]. Flow cytometry (FC) of fine needle aspirates (FNA) has been increasingly applied as first-line analysis in cases of suspected lymphoma in dogs (Riondato and Comazzi 2021) [13]. FNA of lymph node was collected in normal saline and analyzed in flow cytometry with CD21 and CD3 monoclonal antibodies for immunophenotyping which showed 69.35% of aspirated cells were tagged with CD21 marker which indicated B type lymphoma (Fig. 5). FC cannot provide a definitive diagnosis of B-cell lymphoma if there were no evidence of neoplastic phenotypic aberrances and/or a high percentage of cells with the same phenotype and in the absence or co-expression of T- and B-cell markers. In these cases, histopathology and immunohistochemistry were gold standard technique to confirm lymphoma (Riondato and Comazzi 2021) [13].

Treatment

The animal was treated as per University of Wisconsin-Madison modified CHOP-25 protocol. It was multidrug protocol containing C for Cyclophosphamide, H for Hydroxy doxorubicin, O for Oxy vincristine and P for Prednisolone as a palliative therapy were given weekly with 4 weeks interval after 1 cycle of medicines were given as per Garrett *et al*. 2002 [14]. The animal was regularly monitored for hematological and biochemical values before every therapeutic regime (Table 2 and 3). Due to lymphoma, increased level of total and direct bilirubin was recorded and the dog was stabilized by giving heptatonic and isotonic fluids for a week. After the dog became stable, it was treated with vincristine at the dose rate of 0.7mg/m^2 of body surface area and advised to give prednisolone orally at the dose rate of 2mg/kg body weight. Usually, dogs were advised to stay for a minimum of 3 hours following chemotherapy on all prescribed days. After one week, the dimensions of peripheral lymph nodes were very much reduced (Table 1) which indicated complete remission after first dose of chemotherapy. Hematology and serum biochemistry were done and they were found to be normal. Again, the dog was treated with second dose of chemotherapy, cyclophosphamide at the dose rate of 250 mg/m^2 body surface area and prednisolone 1.5 mg/kg body weight. After 2 days, dog was restless with severe vomiting and diarrhea. Hematology revealed anemia, thrombocytopenia, neutropenia (Table 2). Increased level of Alkaline phosphatase (ALP) might be due to hepatic involvement, as well as previous exposure to glucocorticoids, but were nevertheless not predictive for treatment response (Wiedemann *et al*. 2005) [15] (Table 3). Urinalysis was also done which revealed presence of bile salts in urine which further confirmed the condition. Inspite of regular monitoring and treatment, animal was collapsed.

Postmortem Examination

Gross examination revealed petechial haemorrhage in the subcutaneous tissue, rounded heart with epicardial haemorrhagic streaks (Fig. 6) and hepatomegaly (Fig. 7). Microscopic examination of lymph node showed large neoplastic pleomorphic lymphoid cells with nuclei two times the size of red blood cells. Centroblastic type of cells were seen which was characterized by presence vesicular nuclei with fine to granular chromatin contained multiple nucleoli and moderate to scanty basophilic cytoplasm with distinct border (Fig. 8). Effacement of neoplastic cells (Fig. 9) which caused complete loss of lymph node architecture as described earlier by Valli *et al*., 2013 [16] and classified as diffuse large B cell lymphoma. Thinning of capsule and thickening of arterioles with congestion were noticed. Neoplastic cells filled the medullary cords with sinus compression. Since, the dog was in stage 'IV' and substage 'a', metastasis was recorded in heart, kidney, testis and lungs. In the heart, multifocal infiltration of neoplastic lymphoid cells in between the myocardial fibers were observed (Aupperle *et al*., 2007) [17] (Fig. 10). Kidney was a common metastatic site for lymphoma (Taylor *et al*., 2019) [18]. Kidney revealed multifocal neoplastic lymphoid cells interstitially infiltrated (Fig. 11). In testis, clusters of neoplastic lymphoid cells were observed inbetween the seminiferous tubules (Fig. 12). In lungs, tumor emboli were noticed in the artery (Fig. 13).

Table 1: Values Obtained by Measuring Lymph Nodes Using Vernier Caliper before and after treatment

Lymph Node	Before Chemotherapy (in mm)						After Chemotherapy (in mm)					
	LEFT			RIGHT			LEFT			RIGHT		
	Cranial-caudal (CC)	Ventral-dorsal (VD)	Medial-lateral (ML)	Cranial-caudal (CC)	Ventral-dorsal (VD)	Medial-lateral (ML)	Cranial caudal (CC)	Ventral-dorsal (VD)	Medial-lateral (ML)	Cranial-caudal (CC)	Ventral-dorsal (VD)	Medial-lateral (ML)
Submandibular	45	35	21	42	35	23	12	7	6	15	8	5
Pre scapular	64	41	35	64	42	35	24	15	10	26	17	11
Axillary	23	15	10	22	15	11	10	6	4	9	7	4
Inguinal	41	36	25	47	39	25	24	15	9	26	16	10
Popliteal	45	41	35	45	42	35	15	10	6	12	8	5

Table 2: Hematological parameters before and after chemotherapy

Parameters	Unit	Normal range	Before Chemotherapy	Before Vincristine	One week after vincristine	2 days after Cyclophosphamide
Hb	g/dL	1	11.4	15	7.8	5.7
PCV	%	35-57	33.4	40.1	22.3	16.8
RBC	10^6/mm^3	4.95-7.87	5.88	6.25	3.94	2.98
WBC	10^3/ mm^3	5-14	7.8	7.9	21	2.3
Platelet	10^5/ mm^3	2.1-6.2	2.81	2.51	3	0.95
Diff. count						
N	%	58-85	75	74	85	82
L	%	27-36	20	20	10	13
M	%	2-10	5	5	5	4
E	%	0-9	0	1	0	1
B	%	0-1	0	0	0	0

Table 3: Serum biochemical parameters before and after chemotherapy

Parameters	Unit	Normal range	Before Chemotherapy	Before Vincristine	One week after vincristine	2 days after Cyclophosphamide
BUN	mg/dL	10-28	9.15	11.56	16.88	19.48
Creatinine	mg/dL	0.5-1.5	1.25	0.88	0.59	0.91
TP	g/dL	5.4-7.1	8.3	7.2	6.4	5.7
Albumin	g/dL	2.3-3.8	3.2	2.4	2.1	2.56
ALT	IU/L	21-102	42	37	33	30
GGT	IU/L	1.4-6.4	5	8	9	12
ALP	IU/L	20-156	216	74	97	924
T Bilirubin	mg/dL	0.15-0.5	2.93	0.96	0.73	0.68
D Bilirubin	mg/dL	0.06-0.12	2.56	0.58	0.65	0.53
Glucose	mg/dL	60-111	120	128	145	131
Cholesterol	mg/dL	135-270	160	144	268	256
Sodium	mmol/L	141-152	110	115	139.8	142.1
Potassium	mmol/L	4.4-5.4	5.68	4.79	4.21	3.98
Chloride	mmol/L	105-115	111	115	108.7	106.8
CK	IU/L	1.2-28	21	24.5	99	89
LDH	IU/L	45-223	224	175	178	158
Calcium	mg/dL	9-11.3	10.92	8.95	10.24	12.13
Phosphorus	mg/dL	2.6-6.2	7.62	4.56	4.68	3.66

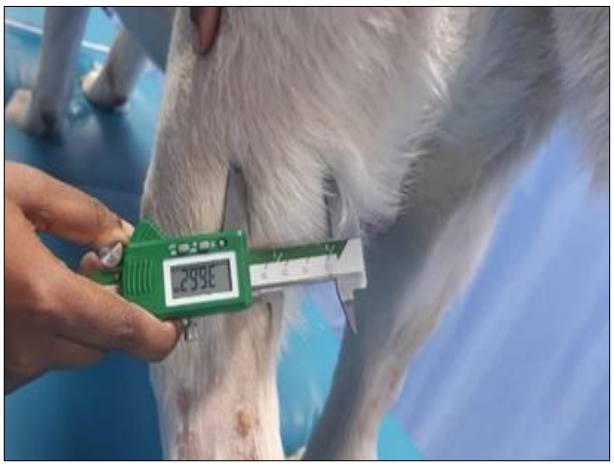

Fig 1: Measurement of popliteal lymph node using vernier caliper

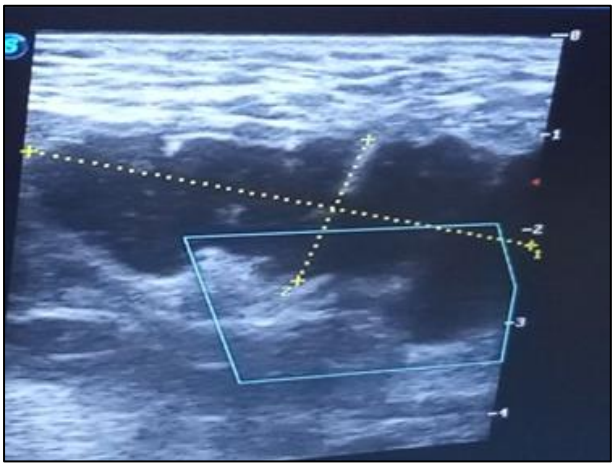

Fig 2: Ultrasonography – measuring the inguinal lymph node

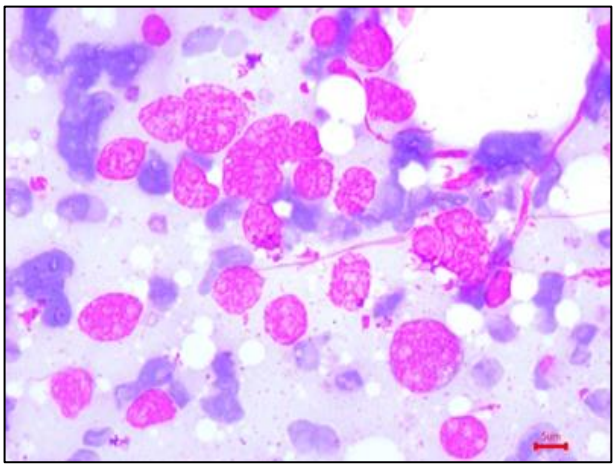

Fig 3: FNAC lymph node Anisocytosis, anisokaryosis, multiple nucleoli with coarse chromatin

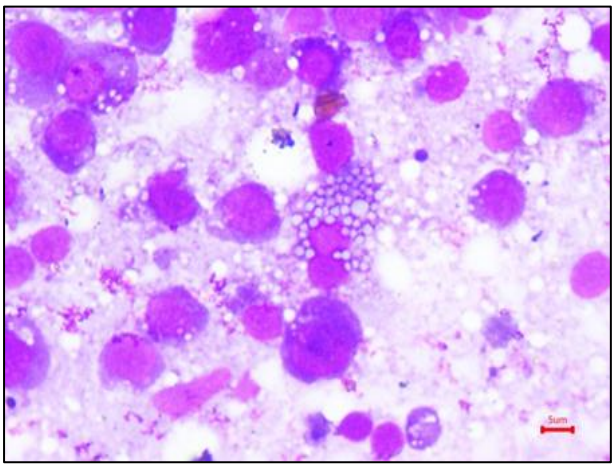

Fig 4: FNAC – lymph node - Russell bodies in a plasma cell (Mott cell)

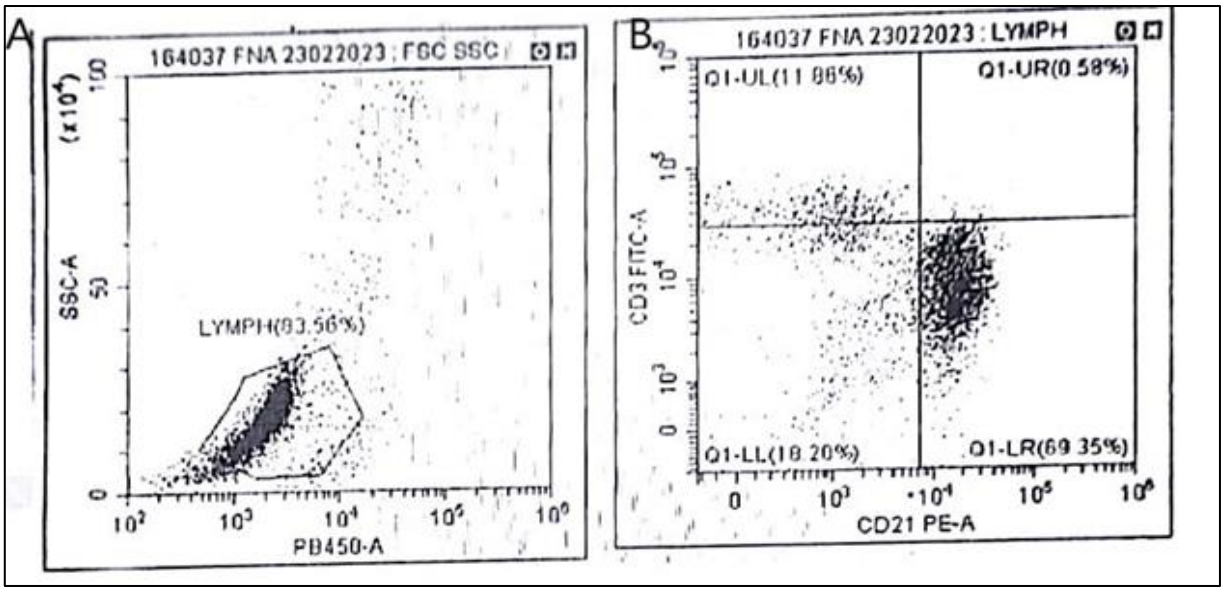

Fig 5: Flowcytometric study: FNA of lymph node: **(A)** Forward scatter (FSC) vs. side scatter (SSC) plot after doublet exclusion showing lymphocytes. **(B)** CD3 vs. CD21 plot of CD45-gated cells showing higher CD21 expression on large cells – 69.35% (Q6-LR) compared with CD3 expression – 11.86% (Q1-UL).

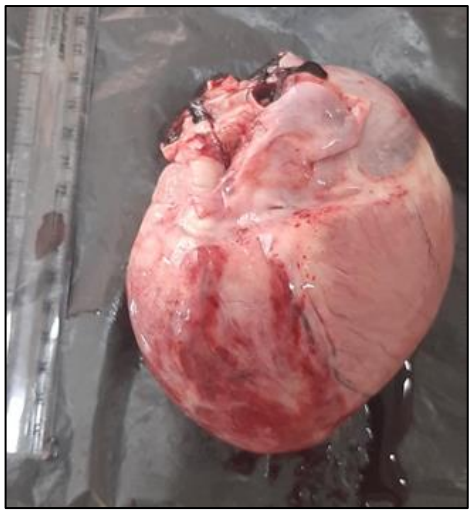

Fig 6: Gross – Heart – Rounded appearance and epicardial haemorrhage

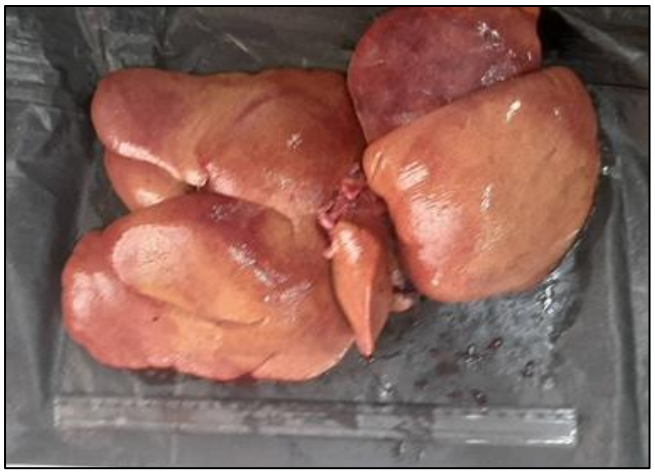

Fig 7: Hepatomegaly with rounded borders and yellowish discoloration

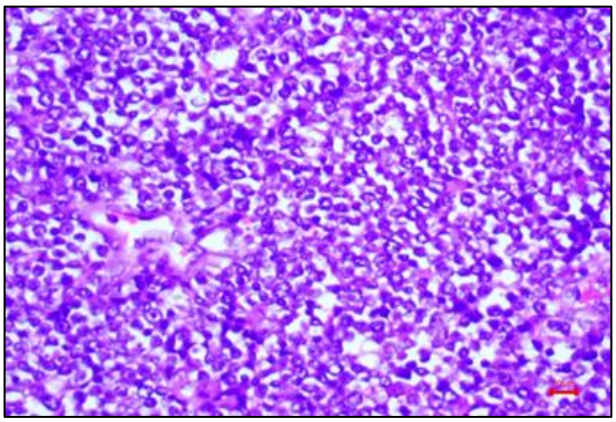

Fig 8: Lymph node – Medium to large sized neoplastic lymphoid cells and fine to coarse chromatin

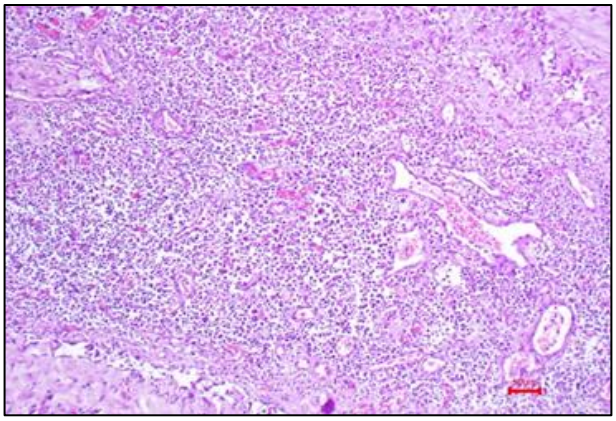

Fig 9: Effacement of nodal structure and neoplastic lymphoid cells filled the lymph node

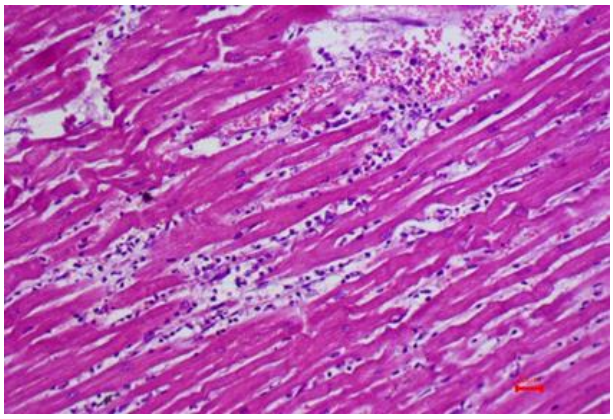

Fig 10: Neoplastic cells infiltrated in between the myocardial fibers.

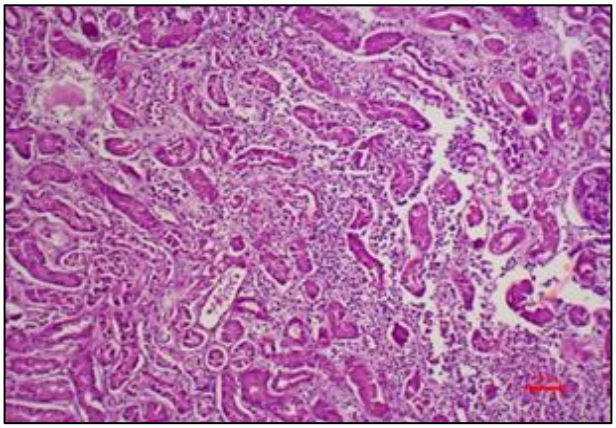

Fig 11: Kidney – Interstitial infiltration of neoplastic lymphoid cells

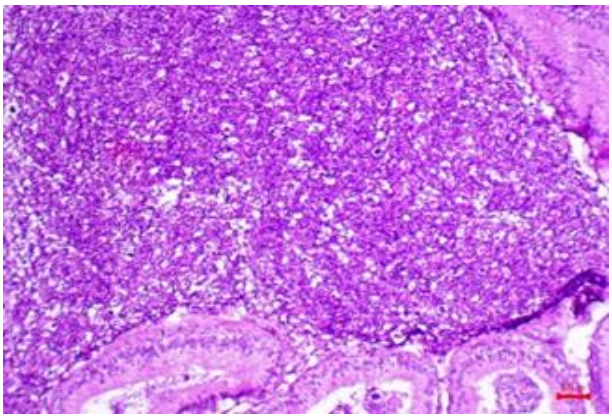

Fig 12: Neoplastic lymphoid cells in seminiferous tubules of testis

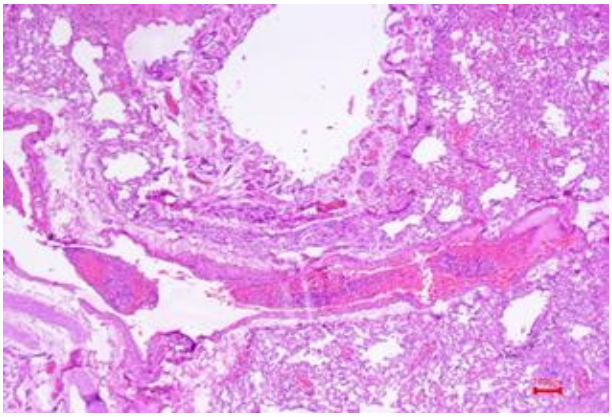

Fig 13: Lung – Tumor emboli

Conclusion

In the present case, animal was died suddenly with abrupt hematological changes (Table 2) might be immune mediated or anaphylactic shock. Increase in ALP which might be due to liver insufficiency. Cyclophosphamide was an alkylating agent that acts by cross-linking strands of DNA, thus preventing DNA replication and cell division. It might cause myelosuppression and gastrointestinal toxicity in some patients and an unusual adverse effect of cyclophosphamide was sterile haemorrhagic cystitis (Dobson 2014) [19] but not noticed in the present case.

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