Japanese Journal of Veterinary Research 66(4): 227–238, 2018

JJVR

REGULAR PAPER Regional Study

Clinical features, haematologic parameters, blood serum biochemistry results and thymidine kinase activity of dogs affected by malignant lymphoma in Turkey

Abdullah Kayar[1,*], Banu Dokuzeylul[1], Onur Iskefli[1], Alper Bayrakal[1], Sinem Ulgen Saka[1], Funda Yildirim[2] and Aydın Gurel[2]

[1] Istanbul University, Faculty of Veterinary Medicine, Department of Internal Medicine, Avcılar-Istanbul / TURKEY
[2] Istanbul University, Faculty of Veterinary Medicine, Department of Pathology, Avcılar-Istanbul / TURKEY

Received for publication, August 25, 2017; accepted, April 3, 2018

Abstract

Canine malignant lymphoma is one of the most common malignant tumours occurring in dogs. Most dogs with malignant lymphoma are characterized by painless peripheral lymphadenopathy. Nonspecific signs of systemic involvement include; lethargy, anorexia, fever, anaemia and weight loss. The mean age of dogs with malignant lymphoma varies from 6.3 to 7.7 years, with no obvious gender predilection. The purpose of this study was to describe in detail clinical features, the haematological and blood serum biochemistry as well as the thymidine kinase activity in canine malignant lymphoma in order to facilitate early diagnosis. Based on cytopathological analysis, canine malignant lymphoma was diagnosed in 20 dogs (11 males and 9 females). The animals' age ranged from 3 to 12 years (median 7.6 years). In 35% of the dogs, regional or general lymphadenopathy was the only clinical sign. In the remaining cases, at least one abnormality connected to canine malignant lymphoma was found. Between ill and healthy dogs, a p-value of < 0.001 was calculated for the haematological parameters: red blood cell count, haemoglobin, haematocrit, platelet, neutrophils and monocytes; for biochemical parameters the following p-values were calculated: blood urea nitrogen $P < 0.01$; aspartate aminotransferase, alkaline phosphatase and thymidine kinase (TK) $P < 0.001$, and Calcium $P < 0.05$. The study results show that in medium and advanced-aged anaemic dogs with non-specific clinical features but at least one enlarged lymph node and elevated ALP and TK concentrations, canine malignant lymphoma must be considered as a possible cause.

Key Words: canine, diagnosis, malignant lymphoma, tumour marker, thymidine kinase (TK).

Introduction

Canine malignant lymphoma is a lymphoproliferative disorder deriving from a clonal proliferation of malignant lymphocytes. The terms lymphoma, malignant lymphoma and

*Corresponding author: Abdullah Kayar, Istanbul University, Faculty of Veterinary Medicine, Department of Internal Medicine, 34320, Avcılar, Istanbul-TURKEY
Fax: +90 212 473 72 41. E-mail: kayar@istanbul.edu.tr
doi: 10.14943/jjvr.66.4.227

lymphosarcoma are understood to mean a tumour of the lymphoid system originating from solid organs (e.g. lymph nodes, spleen, liver) and which is differentiated from those originating in the bone marrow, defined as lymphoid leukaemia[2].

"Canine malignant lymphoma" is a term which the internationally accepted. But the terms "canine lymphoma" and "canine lymphosarcoma" are used synonymously in different countries[2,29].

Lymphoma is a commonly occurring, spontaneous neoplasm in dogs, accounting for approximately 8 to 10 percent of all canine tumours[3,7,23] and with a reported annual incidence rate of at least 24 to 33 per 100.000 dogs[4,13,31,36].

Most dogs with malignant lymphoma are characterized by nonpainful peripheral lymphadenopathy[4,31,32]. The disease is often characterized by hepatosplenomegaly and in most cases is aleukemic[31]. Non-specific signs of systemic involvement include; lethargy, anorexia, fever, anaemia, loss of endurance, and weight loss[12,18].

The multicentric form of the disease is the most commonly found haematopoietic neoplasm in dogs, with varying degrees of infiltration of the lympho-reticular system, including lymph nodes, liver, spleen, blood and bone marrow in which it accounts for 84% of all cases of lymphoma[3,28,30]. In the early stages of multicentric lymphoma, clinical signs may be mild, and 80% of dogs with lymphoma are presented with superficial, painless, generalized lymphadenopathy[9,19]. Radiological and ultrasonographic examinations also play an important part in the initial assessment of the case[1].

Canine malignant lymphoma and leukemia are consistently fatal diseases, but early diagnosis and an appropriate chemotherapeutic intervention can increase the survival time of patients[26]. The diagnosis of canine malignant lymphoma and leukemia is based on histopathological and cytological findings: as a result, establishing diagnoses in cases where tissue samples for histopathological examination are not available is a challenge. In these cases, a non-invasive diagnostic procedure such as plasma thymidine kinase activity can be very helpful[21].

Many studies have been carried out on various aspects of canine malignant lymphoma such as; clinical features, its haematology, blood serum biochemistry, cytopathology and thymidine kinase activity. In this study, these aspects have been examined synoptically in detail and compared with the individual results of previous research in order to evaluate parameters for diagnostic marker of the disease.

Materials and Methods

Control group: A total of twenty dogs, comprising clinically healthy, client-owned dogs that underwent regular examinations (including general health checks and/or regular vaccinations between July 2015 and May 2016), had no treatment for neoplasia or a serious non-neoplastic disease in the past or recent past in their anamnesis and had normal results of physical examination, hemogram, blood serum biochemistry, thymidine kinase, radiography and ultrasonography, admitted to the Department of Internal Medicine, Faculty of Veterinary Medicine, Istanbul University, served as control group. Their information regarding signalment is presented in Table 1.

Animals with non-neoplastic diseases were presented with a variety of clinical signs; they had no evidence of malignancy based on results of physical examination, blood work, thoracic radiography and/or abdominal ultrasound. All dogs were subjected to thoracic and abdominal radiography and/or abdominal ultrasonography, and the following analyses were carried out: Complete Blood Count (CBC), leucocyte differential count (LDC) (neutrophil, eosinophil, basophil granulocytes, lymphocyte, monocyte agranulocytes), blood serum biochemical profile (glucose, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase

Table 1. Summary of clinical data for 40 dogs

Case Number	Breed	Gender	Age (yrs)	Stage / Substage	Diagnosis
1	Golden Retriever	M	7	IIIb	Malignant Lymphoma
2	French Bulldog	M	5	IVb	Malignant Lymphoma
3	White Coated Terrier	F	3	IVb	Malignant Lymphoma
4	White Coated Terrier	F	12	IIIb	Malignant Lymphoma
5	Beagle	M	9	IIIa	Malignant Lymphoma
6	Yorkshire Terrier	FS	7	IIIb	Malignant Lymphoma
7	Golden Retriever	M	12	IIIb	Malignant Lymphoma
8	Mixed breed	FS	6	IIIa	Malignant Lymphoma
9	Golden Retriever	M	9	IIIa	Malignant Lymphoma
10	White Coated Terrier	F	11	IIIb	Malignant Lymphoma
11	American Cocker Spaniel	FS	11	IIIa	Malignant Lymphoma
12	Golden Retriever	FS	5	IIIa	Malignant Lymphoma
13	Rottweiler	M	9	IIIa	Malignant Lymphoma
14	Turkish Karabash	M	6	IIIb	Malignant Lymphoma
15	Rottweiler	M	6	IIa	Malignant Lymphoma
16	Pitbull Terrier	FS	8	IIIb	Malignant Lymphoma
17	German Shepherd	M	5	IIIb	Malignant Lymphoma
18	American Staffordshire Terrier	M	5	IIIb	Malignant Lymphoma
19	White Coated Terrier	MC	9	IIIb	Malignant Lymphoma
20	Golden Retriever	FS	6	IIIb	Malignant Lymphoma
21	White Coated Terrier	M	7		Healthy
22	German Shepherd	M	3		Healthy
23	American Cocker Spaniel	FS	6		Healthy
24	Yorkshire Terrier	M	4		Healthy
25	Turkish Karabash	M	5		Healthy
26	Golden Retriever	F	5		Healthy
27	German Shepherd	F	5		Healthy
28	Beagle	M	5		Healthy
29	White Coated Terrier	FS	10		Healthy
30	American Cocker Spaniel	M	7		Healthy
31	German Shepherd	FS	8		Healthy
32	White Coated Terrier	FS	7		Healthy
33	French Bulldog	MC	9		Healthy
34	Mixed breed	F	5		Healthy
35	Golden Retriever	FS	6		Healthy
36	Golden Retriever	M	7		Healthy
37	White Coated Terrier	FS	5		Healthy
38	Rottweiler	M	7		Healthy
39	Rottweiler	M	5		Healthy
40	Golden Retriever	M	6		Healthy

F: Female, FS: Female Spayed, M: Male, MC: Male Castrated

(ALP), gamma glutamyl transferase (GGT), cholesterol, calcium (Ca) concentrations and thymidine kinase (TK) activities. The dogs were considered healthy after normal physical examination, haematology, biochemistry, thymidine kinase activity, radiography and ultrasonography results. Animals with diseases or abnormalities in the tests performed were excluded from the control group.

CBCs were performed on a Mindray BC-2800 Vet Auto Hematology Analyzer (Mindray Bio-Medical Electronics Co., Ltd. Shenzhen / China) with whole blood in ethylenediaminetetraacetic acid (EDTA) anti-coagulant. Blood serum biochemical analyses were performed using fresh serum. Serum biochemical concentrations were determined using Spinreact S.A.U. and Cormay S.A. Kits in an Prestige 24i autoanalyser (Tokyo Boeki, Medical System, Tokyo, Japan). Serum samples were stored at −20°C and used for TK activity assay within 3 weeks after collection. The TK activity in serum was determined using a ^{125}I-based radioenzyme assay as previously described. The reference interval (normal 0–7 U/L) was derived from a previous veterinary study[33].

Canine malignant lymphoma group: Twenty dogs affected by malignant lymphoma were presented to the Department of Internal Medicine, Faculty of Veterinary Medicine, University of Istanbul, between January 2014 and July 2016. Information regarding signalment, stage and substage is presented in Table 1.

Only dogs with enlarged peripheral lymph nodes and without signs of concurrent diseases, localized infections or a history of previous chemotherapy or prednisolone treatment were included in the study. All patients were subjected to a clinical examination and CBC, LDC, serum biochemical profile and TK test. Thoracic and abdominal radiography and/or abdominal ultrasonography were performed in all dogs after physical examination with owner consent. Staging was based on the modified World Health Organization (WHO) staging system for canine malignant lymphoma. Dogs were also classified as WHO substage “a” or “b” depending on whether they were clinically well of suffering from clinical signs of their lymphoma at presentation, respectively.

Fine-needle aspiration biopsies were taken from palpable lymph nodes (submandibular, prescapular, popliteal) and sent to the Pathology Department. The cytological examinations (based on air dried and stained with May-Grünwald Giemsa smear technique) were performed by two pathologists working independently. Cytomorphologic features of malignancy for lymphomas were adopted from a previous study[27]. According to the study, the criteria were based on the cell size (“small,” “medium,” or “large,” i.e., nuclei smaller than, equal to, or larger than 2 red blood cells); the shape of the nuclei; the density of the chromatin; the number, size, and distribution of the nucleoli; and the volume and basophilia of the cytoplasm. The cases showing features as irregular nuclear outlines, macronuclei, irregular clumped chromatine, multinucleoli, high nuclear/cytoplasmic ratio and abundant pale cytoplasms were decided as malignant lymphomas.

Statistical analysis: The Mann-Whitney U test was used for inter-group comparison. The statistical analysis of the data was carried out with the SPSS software 11.0. (IBM Corporation, USA). $P < 0.05$ was considered significant for all tests.

Results

Control group

The animals’ mean age was 6.1 (range 3–10) years, and the group consisted of 11 males (10 intact and 1 castrated) and 9 females (3 intact and 6 spayed). Ten breeds were represented in this study: White Coated Terriers and Golden Retrievers (with 4/20; 20%, each), German Shepherds (with 3/20; 15%), American Cocker

Table 2. Occurrence of clinical signs other than lymphadenomegaly in dogs affected by malignant lymphoma

Clinical Signs	% of dogs
Non-specific systemic clinical signs (diminished appetite, general weakness, lethargy)	60
Paleness of mucosal membranes	55
Diarrhoea	20
Hind legs oedema	20
Dyspnea	15
Neck oedema	15
Testis oedema	15
Dermatological lesions (rash, eczematous lesions)	15
Vomitus	10
Splenomegaly	10
Ascites	10
Temporary blindness	10
Bilateral retinal detachment, uveitis	10
Oedema on the ventral breast section	5
Recurring or chronic fever	5
Exophthalmos	5
Corneal oedema	5

Spaniels and Rottweilers (with 2/20; 10%, each), Turkish Karabash, Beagle, French Bulldog, Mixed breed and Yorkshire Terrier (with 1/20; 5%, for each of these breeds). The median body weight was 26 kg (range, 3.5–42 kg). Full haematology and serum biochemistry were detected within normal limits. Serum TK activities were 1–6 U/L (normal < 7).

Canine malignant lymphoma group

Twenty dogs with malignant lymphoma were included. The animals' mean age was 7.6 (range 3–12) years, and the group consisted of 11 males (10 intact and 1 castrated) and 9 females (3 intact and 6 spayed). Twelve breeds were represented in this study: The most frequently encountered breeds were Golden Retrievers (5/20; 25%) and White Coated Terriers (4/20; 20%) and Rottweilers (2/20; 10%), German Shepherd, French Bulldog, Beagle, Mixed breed, American Cocker Spaniel, Turkish Karabash, Pitbull, Yorkshire Terrier and American Staffordshire Terrier (with 1/20; 5%, for each of these breeds). All dogs were categorized as multicentric form. When considering clinical stage, 1 dog had stage II disease (substage a), 17 dogs had stage III disease (6 substage a and 11 substage b) and 2 dogs had stage IV disease (substage b) (Table 1).

Clinical signs in canine malignant lymphoma group

In 35% of dogs, the regional or general lympadenopathy was the only clinical sign, in the remaining cases (65%), at least one aberration connected to canine malignant lymphoma was found. While only one animal was diagnosed with regional lymphadenopathy, generalised lymphadenopathy was identified in all the other dogs. Clinical signs and their occurrence in the examined dogs are presented in Table 2.

Clinicopathologic findings: Haematological and biochemical abnormalities

The most common clinicopathologic abnormalities at the time of initial examination were anaemia (11/20 [55%]), leukocytosis (6/20

Table 3. Haematological findings (total group size: 20 dogs with malignant lymphoma)

Haematocrit	n =	Percentage
21–30 %	10	50.0
31–37 %	1	5.0
> 37 %	9	45.0
Leucocytes		
Leukopenia (< 6 × 10^3μL)	1	5.0
Normal (6–17 × 10^3μL)	13	65.0
Leucocytosis (> 17 × 10^3μL)	6	30.0
Thrombocyte		
Thrombocytopenia (< 200 × 10^3μL)	7	35.0
Normal (200–500 × 10^3μL)	13	65.0
Thrombocytosis (> 500 × 10^3μL)	–	–
Lymphocytes		
Lymphopenia (< 8%)	1	5.0
Normal (8%–21%)	16	80.0
Lymphocytosis (> 21%)	3	15.0

[30%]) and thrombocytopenia (7/20 [35%]) (Table 3). Bone marrow evaluation of the dogs was not performed. Twelve dogs (60%) had high ALP activity, 10 (50%) high serum AST activity and 5 (25%) high serum GGT activity. The level of serum TK activity in 20 dogs with malignant lymphoma ranged from 8.9 to 470 U/L. All dogs with malignant lymphoma have been shown to have a 1.3–67 times higher serum TK activity than normal dogs. The p-value for anaemia, PLT, neutrophils, monocytes, AST, ALP, TK between the control group and the canine malignant lymphoma group was $P < 0.001$. The BUN concentration was higher in the canine malignant lymphoma group than in the control group ($P < 0.01$). A statistically significant difference was found between the control group and the canine malignant lymphoma group with respect to Ca ($P < 0.05$). No statistically significant difference was found between the control group and the canine malignant lymphoma group with respect to WBC, MCV, MCH, MCHC, eosinophils, basophils, lymphocytes, glucose, creatinine, ALT, GGT and cholesterol ($P > 0.05$) (Table 4). All normal range of haematological and biochemical value, according to manufacturer's recommendation: RBC 5.5–8.5 (× 10^6μL), HGB 12–18 (g/dl), HCT 37–55 (%), WBC 6–17 (× 10^3μL), PLT 200–500 (× 10^3μL), MCV 60–77 (fL), MCH 19.5–26 (pg), MCHC 32–36 (g/dl), Neutrophil 40–80 (%), Eosinophil 1–6 (%), Basophil 0–1 (%), Lymphocyte 20–40 (%), Monocyte 2–10 (%), Glucose 60–125 (mg/dl), BUN 7–27 (mg/dl), Creatinine 0.5–1.6 (mg/dl), AST 5–55 (IU/L), ALT 5–60 (IU/L), ALP 10–150 (IU/L), GGT 0–10 (IU/L), Cholesterol 112–328 (mg/dl), Ca 8.9–11.4 (mg/dl), TK 1–6 (U/L).

Cytopathological findings

Based on their cytopathological findings, the 20 dogs were confirmed malignant lymphoma. Most of the cells in cytology smears were lymphoblasts with deep blue granular scanty cytoplasm and multiple nucleoli. Anisocytosis, anisokaryosis, macronuclei and multiple nucleoli were identified (Figs. 1A and 1B). Many basophilic glandular bodies, fragile and degenerated lymphoblastic cells and abnormal mitotic figures were observed in some cases (Figs. 1C and 1D). Small sized, cleaved lymphocytes having round and slightly irregular nuclei, fine chromatine and scant and pale basophilic cytoplasm were also

Table 4. Results of clinicopathologic testing in 40 dogs with malignant lymphoma and healthy dogs

Parameter	Canine Malignant Lymphoma (n = 20)		Healthy (n = 20)		
	Mean ± SE	Range	Mean ± SE	Range	
RBC ($\times 10^6\mu$L)	4.99 ± 0.33	2.97–7.48	7.04 ± 0.23	5.35–9.30	***
HGB (g/dl)	10.92 ± 0.74	6.7–17.1	15.62 ± 0.57	11.2–19.1	***
HCT (%)	34.63 ± 2.05	21–52	48.79 ± 1.57	37–58	***
WBC ($\times 10^3\mu$L)	19.59 ± 5.13	5.3–91.7	11.43 ± 0.89	5.6–18.7	N.S.
PLT ($\times 10^3\mu$L)	233.63 ± 24.92	62–460	369.42 ± 20.98	224–518	***
MCV (fL)	70.32 ± 0.95	63–77	69.37 ± 0.85	60–75	N.S.
MCH (pg)	22.11 ± 0.40	18–25	22.26 ± 0.25	20–24	N.S.
MCHC (g/dl)	31.53 ± 0.41	29–36	31.89 ± 0.30	30–34	N.S.
Neutrophil (%)	77.11 ± 4.35	1–87	65.11 ± 1.18	54–79	***
Eosinophil (%)	2.79 ± 0.34	0–6	3.00 ± 0.32	1–6	N.S.
Basophil (%)	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	N.S.
Lymphocyte (%)	17.11 ± 4.70	5–99	13.74 ± 0.90	8–21	N.S.
Monocyte (%)	2.79 ± 0.24	0–5	5.63 ± 0.46	3–9	***
Glucose (mg/dl)	113.89 ± 5.31	80–153	108.32 ± 2.76	97–143	N.S.
BUN (mg/dl)	41.53 ± 6.55	13–136	24.05 ± 2.61	9–54	**
Creatinine (mg/dl)	0.83 ± 0.05	0.4–1.2	0.84 ± 0.05	0.4–1.2	N.S.
AST (IU/L)	67.21 ± 8.59	24–150	26.79 ± 1.38	17–40	***
ALT (IU/L)	75.42 ± 17.98	16–333	37.53 ± 3.53	24–87	N.S.
ALP (IU/L)	546.68 ± 200.47	20–3752	86.32 ± 10.30	34–168	***
GGT (IU/L)	9.95 ± 2.01	1–36	5.05 ± 0.42	3–9	N.S.
Cholesterol (mg/dl)	241.47 ± 22.95	117–553	224.16 ± 14.17	118–338	N.S.
Ca (mg/dl)	10.10 ± 0.36	6.4–12.4	9.35 ± 0.24	7.9–11.5	*
TK (U/L)	85.21 ± 24.67	8.9–470	3.32 ± 0.43	1–6	***

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, BUN: Blood urea nitrogen, Ca: Calcium, GGT: Gamma glutamyl transferase, HCT: Haematocrit, HGB: Haemoglobin, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, PLT: Platelet, RBC: Red blood cell count, TK: Thymidine kinase, WBC: White blood cells
N.S. $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

determined in a few cases.

Discussion

Many studies[5,15,22,25,35] providing diagnostic and prognostic information to clinicians in veterinary medicine were carried out on dogs with malignant lymphoma. Various markers such as clinical utility, hematology, blood serum biochemistry profile, cytopathology and thymidine kinase activity were examined in these studies.

Sapierzyński *et al.*[25] reported that, regional or general lymphadenomegaly was the sole clinical feature, and it was found only in 29% of diseased dogs, while the remaining 71% suffered from at least one lymphoma-related health problem with non-specific systemic clinical signs (diminished appetite, general weakness), recurring or chronic fever, dyspnea, paleness of mucosal membranes, dermatologic lesions, hind legs oedema, temporary blindness. The animals' age ranged from 1.5 year to 15 years (median 7.5 years). In a study involving 46 cases of canine malignant lymphoma at the diagnosis stage, Fournel-Fleury *et al.*[6] reported that the most

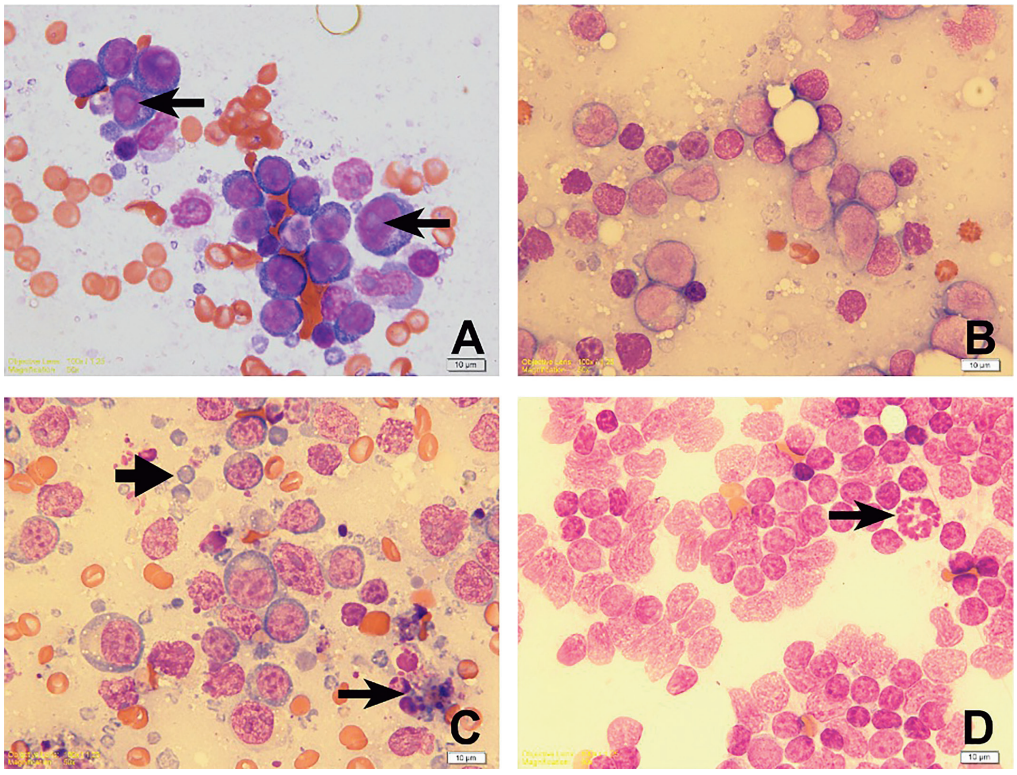

Fig. 1A. Lymphoblastic cells with prominent and large nucleoli (arrows), basophilic cytoplasm. MGG stain, Bar = 10 μm. **Fig. 1B. Lymphoid cells.** Atypical, small, medium, and large cells displaying considerable nuclear pleomorphism, scant and lightly basophilic cytoplasm, MGG stain, Bar = 10 μm. **Fig. 1C. Predominantly large cells with multiple prominent nucleoli.** Numerous lymphoglandular bodies on the background (thick arrow) and a tangible body macrophage (thin arrow). MGG stain, Bar = 10 μm. **Fig. 1D. Moderate sized, round or irregular nuclei with coarse chromatin and degenerated cells, an irregular mitotic figure (arrow).** MGG stain, Bar = 10 μm.

frequent finding was generalised lymphadenopathy (28/46), while regional lymphadenopathy was identified in only 16 cases. On the other side, Sapierzyński and Micuń[24] found that commonly a general type of lymphadenomegaly occurred in affected dogs while the regional form was rather rare (The average of dogs examined was 8 years). In the present study, similarly to the results of Sapierzyński *et al.*[25], while we found regional or generalised lymphadenopathy as the only clinical sign in 35% of our cases in the 3–12 age range with a mean age of 7.6 years, we found at least one problem connected to lymphoma in 65% of them. At first clinical examination of our cases, regional lymphadenopathy was found in only one case while generalized lymphadenopathy was found in all remaining cases. This clinical sign found is compatible with the result of Sapierzyński and Micuń[24] that generalized lymphadenomegaly usually occurs in the lymphoma in dogs. On the other hand, the fact that generalized lymphadenopathy was found at higher rates compared to Fournel-Fleury *et al.*[6] can be attributed to the late referrals of patients to the Faculty Hospital.

In the initial assessment phase of 84 dogs (ranged from 1.7 year to 13.9 years, median: 8 years) with malignant lymphoma, Miller *et al.*[16] identified anaemia with a mean packed cell volume (PCV) of 35% (range 25–39%) in 27 dogs (32%). For 96% of the cases (26/27), they reported normocytic, normochromic anaemia, along with microcytic, normochromic anaemia in only one case (4%). According to the study, cancer-related anaemia is typically normocytic, normochromic. It has been reported by Gavazza *et al.*[8] that

48.3% of dogs (ranged from 1.8 year to 15 years) with malignant lymphoma develop anaemia, of which 62.1% are normocytic, normochromic with haematocrit values in the range of 30–37%, which in most cases takes a mild course (81%). In the present study, moderate anaemia between 21–30% was found in 50% of the cases while mild anaemia between 31–37% was found in 5% of them (Table 3). The anaemia rate found was higher than the rate of Miller *et al.*[18] but close to the results of Gavazza *et al.*[8]. In our study, normocytic, normochromic type of anaemia was found as the cancer-related anaemia in all anaemic cases, similarly to the results of Miller *et al.*[16]. The determination anaemia of hemogram analysis a dog suspected lymphoma at the end of clinical examination and we can evaluate this as normocytic normochromic type of anaemia sensitive indicator and positive predictive value.

In a study, carried out by Teske[28] on NHL (Non-Hodgkin Lymphoma)-affected dogs, 49.7% were found to have normal leucocyte counts, while 31.8% suffered from leucocytosis and 18.5% from leukopenia. Madewell[14] on the other hand, identified leukopenia in only 9.3–12.5% of dogs with NHL. In the study carried out by Miniscalco *et al.*[17] on 48 dogs with malignant lymphoma, leucocytosis was detected at varying rates from mild to very severe (18.3–342 $\times$ 10^3μL) in 33% of the cases. In our study, leucocytosis at varying rates (5.3–91.7 $\times$ 10^3μL) in 30% of the cases and leucopenia in 5% of the cases were found.

Thrombocytopenia has been reported[17] in 44% of all dogs suffering malignant lymphoma. In a study on neoplasia-related thrombocytopenia involving 57 dogs with malignant lymphoma[11] a platelet concentration of 120.193 $\pm$ 58.175 /μL was found. In the present study, thrombocytopenia was found in 35% of the cases with an average of 233.63 $\pm$ 24.92 $\times$ 10^3μL while platelets were found to be normal in the remaining 65% of the cases. We can attribute the reason for the fact that we found thrombocytopenia with a lower percentage compared to the researchers despite the cases with an advanced clinical stage to the fact that the number of cases was not high. We may also consider reasons such as increased platelet consumption associated with neoplasia, decreased platelet production, and sequestration of platelets as the reason for thrombocytopenia, as it is indicated by the researchers.

Pavel and Manolescu[22] examined 10 dogs with malignant lymphoma. The LDC yielded the following result: Neutrophils 63–85% and lymphocytes 8–28%. In the study carried out on 48 dogs with malignant lymphoma[17], lymphocytosis and lymphopenia were found in 19% and 25% of the cases, respectively. In the present study, while lymphocytosis and lymphopenia were found in 15% and 5% of the cases, respectively, neutrophils and lymphocytes were found in the ranges of 1–87% and 5–99%, respectively.

Williams *et al.*[34], who studied 51 cases of canine malignant lymphoma (The median age was 7 years range 1–13 years), found hypercalcemia in 3 (6%), high serum ALT concentrations in 14 (27%), and high serum ALP concentrations in 15 dogs (29%). Gavazza *et al.*[8], on the other hand, found 16.7% showed signs of liver damage in their biochemical profile of dogs with malignant lymphoma. In the study carried out by Mutz *et al.*[20] on 77 patients with multicentric lymphoma, hypercalcemia ($>$ 12 mg/dL) was found in 5 cases (6%). Unlike hypercalcemia which was detected in less than 10% of the cases, hypercalcemia was detected in 20–40% of the cases in some studies. Fournel-Fleury *et al.*[6] established the biochemical profile of 46 dogs with malignant lymphoma and found no remarkable changes with the exception of hypercalcemia (in 20–40% of dogs). Greenlee *et al.*[10] stated that about 20% of dogs affected by the disease had a calcium concentration exceeding 11.5 mg/dl. Blood serum biochemistry including BUN, AST, ALP and Ca parameters were highly elevated in dogs with malignant lymphoma in our study. The BUN concentration, however, was higher in the canine malignant lymphoma group than in the control group ($P < 0.01$). In the authors' opinion, this has less to do with renal problems but is a prerenal phenomenon and

related to the dehydration of the dogs. AST was elevated in 9 of the 20 sick dogs (45%), while ALP was elevated in 12 dogs (60%). The serum AST activity, was not spesific and sensitive a marker for malignant lymphoma, because it is elevated with skeletal muscle necrosis, muscle inflammation and damage, exercise, myocardial damage and hepatocellular necrosis. It may be considered that we found a rate of 60% of patients in ALP concentration because it is treated as a diagnostic biomarker in tumoral formations. In the present study, the mean Ca value of our 20 dogs with malignant lymphoma was found to be 10.10 ± 0.36 mg/dl; only 2 dogs had a Ca concentration (12.4 mg/dL and 11.8 mg/dL respectively) exceeding the reference value. While this value conforms to the 10% rate reported in some papers, it is lower than the value found by a few researchers. Furthermore, increased concentration of blood serum Ca and ALP activities on dogs which have lymphoma, not being specific indicators, we can consider these parameters as sensitive indicators, though not being as much as serum TK parameters (because the increase of Ca and ALP did not occur at all patients). Therefore, we are considering that it must be given priority to the evaluation of the measurements of Ca, ALP and TK in biochemical blood parameters at lymphoma suspicious dogs compared to the other parameters.

Nakamura *et al.*[21] found increased plasma TK in all dogs with malignant lymphoma. The team had examined a group of 20 dogs and found a plasma TK activity of 6.8–430 U/l. The animals' age ranged from 3 years to 15 years (median: 7.4 years). Although plasma TK activity seemed to be a sensitive plasma marker for lymphoma and leukemia in their study, most of the cases were in advanced stages (IV to V). If we consider the stages II, III and IV of the dogs having malignant lymphoma (mostly stage III) in our study, we submit that it is important positive predictive value, being high sensitive and serum TK increase of these patients in the evaluations of early stage of canine lymphoma needed significantly.

The researchers[33] reported that a large majority of dogs with malignant lymphoma or even all of them exhibited a TK activity above the reference value range. The mean age was 7.9 years (range: 1–16 years). In their studies they found serum TK values in lymphoma-affected dogs (range 5–900 U/L) whose upper limit was 129 times higher than the value for healthy dogs. In this study, increased serum TK activities (range of 8.9–470 U/L) were detected in all dogs with malignant lymphoma. The difference with respect to the control group had a statistical significance of $P < 0.001$. In the comparison made in terms of TK, the mean age of the dogs in the present study was similar to the mean age of the dogs in the studies of other researchers.

Although cytological examination of fine-needle aspiration biopsy (FNAB) specimens has been generally accepted as a reliable technique for diagnosing malignant lymphoma in the dog, as the researchers[30] stated, there have been no reports on the validity of FNAB technique in classifying lymphomas. In our study we only evaluated malignancy of the lymphomas according to the cytomorphologic features. For detection of the cells' clonality and classification and more precise diagnosing it is needed immunophenotyping.

The decrease in RBC, HGB, HCT and PLT parameters and the increase in WBC parameter are important when we compare the hemogram parameters in dogs with malignant lymphoma with the reference parameters in healthy dogs. In terms of blood serum biochemistry, elevations in ALP, Ca and TK levels are important. We consider the evaluation of RBC, HGB, HCT, WBC, PLT, ALP, Ca and TK parameters primarily when the hemogram and blood biochemical findings are evaluated in diagnostic procedures in suspected dogs with lymphoma clinically. Among these parameters, we assume that it should be considered with particular importance to TK parameter as a parameter with high sensitivity because of the increase in every case.

In conclusion, in this study, the authors have examined in detail various aspects of canine malignant lymphoma such as; clinical features, its haematology, blood serum biochemistry, cytopathology and thymidine kinase activity, and compared their findings with the individual results of previous research in order to evaluate parameters for diagnostic marker of the disease. In the authors' opinion, canine malignant lymphoma must be considered as a possibility in the case of anaemic medium-age and old dogs with non-specific clinical features, at least one enlarged lymph node, and elevated ALP and TK concentrations. Additionally, all of these diagnostic criteria can not be compared in patients with different health situations. After this preliminary work, the authors intend to carry out a more extensive study comprising a larger number of dogs using a canine malignant lymphoma blood test in addition to a cytopathological analysis in the diagnosis. This future study will include the comparison of clinical, haematological, blood serum biochemistry, cytopathological and TK parameters as well as immunophenothypic sub-classification.

Acknowledgments

This work was supported by Scientific Research Projects Coordination Unit of Istanbul University. Project number 58949. The authors would like to thank Dr. Omur KOCAK for the statistical analysis.

References

1) Blackwood L, Sullivan M, Lawson H. Radiographic abnormalities in canine multicentric lymphoma: a review of 84 cases. J Small Anim Pract 38, 62–69, 1997
2) Capurro C, Buracco P, Rossi L. Lymphoma in dogs. Veterinaria 4, 15–27, 1990
3) Dobson JM, Gorman NT. Canine multicentric lymphoma 1: Clinico-pathological presentation of the disease. J Small Anim Pract 34, 594–598, 1993
4) Dorn CR, Taylor D, Hibbard HH. Epizootiologic characteristics of canine and feline leukemia and lymphoma. Am J Vet Res 28, 993–1001, 1967
5) Elliott JW, Cripps P, Blackwood L. Thymidine kinase assay in canine lymphoma. Vet Comp Oncol 11, 1–13, 2013
6) Fournel-Fleury C, Ponce F, Felman P, Blavier A, Bonnefont C, Chabanne L, Marchal T, Cadore JL, Goy-Thollot I, Ledieu D, Ghernati I, Magnol JP. Canine T-cell lymphomas: a morphological, immunological, and clinical study of 46 new cases. Vet Pathol 39, 92–109, 2002
7) Gavazza A, Presciuttini S, Barale R, Lubas G, Gugliucci B. Association between canine malignant lymphoma, living in industrial areas, and use of chemicals by dog owners. J Vet Intern Med 15, 190–195, 2001
8) Gavazza A, Lubas G, Valori E, Gugliucci B. Retrospective survey of malignant lymphoma cases in the dog: clinical, therapeutical and prognostic features. Vet Res Commun 1, 291–293, 2008
9) Gorman NT. The haemolymphatic system. In: Manual of small animal oncology. White RAS, eds. The British Small Animal Veterinary Association (BSAVA), Cheltenham. pp. 207–234, 1991.
10) Greenlee PG, Filippa DA, Quimby FW, Patnaik AK, Calvano SE, Matus RE, Kimmel M, Hurvitz AI, Lieberman PH. Lymphomas in dogs. A morphologic, immunologic, and clinical study. Cancer 66, 480–490, 1990
11) Grindem CB, Breitschwerdt EB, Corbett WT, Page RL, Jans HE. Thrombocytopenia associated with neoplasia in dogs. J Vet Intern Med 8, 400–405, 1994
12) Jarrett WFH, Crighton GW, Dalton RG. Leukemia and lympho-sarcoma in animals and man. Vet Rec 79, 693–699, 1966
13) MacVean DW, Monlux AW, Anderson Jr PS, Silberg SL, Rozsel JF. Frequency of canine and feline tumors in a defined population. Vet Pathol 15, 700–715, 1978
14) Madewell BR. Hematological and bone marrow cytological abnormalities in 75 dogs with malignant lymphomas. J Am Anim Hosp Assoc 22, 235–240, 1986
15) Martini V, Melzi E, Comazzi S, Gelain ME. Peripheral blood abnormalities and bone marrow infiltration in canine large B-cell lymphoma: is there a link? Vet Comp Oncol 13, 117–123, 2015

16) Miller AG, Morley PS, Rao S, Avery AC, Lana SE, Olver CS. Anemia is associated with decreased survival time in dogs with lymphoma. J Vet Intern Med 23, 116–122, 2009
17) Miniscalco B, Guglielmino R, Morello E, Tarducci A, Geuna M. Clinical usefulness of peripheral blood lymphocyte subsets in canine lymphoma. Vet Res Commun 27, 407–409, 2003
18) Moldovanu G, Moore AE, Friedman M, Miller DG. Cellular transmission of lymphosarcoma in dogs. Nature. 210, 1342–1343, 1966
19) Moulton JE, Harvey JW. Tumors of lymphoid and hematopoetic tissue. In: Tumors of Domestic Animals. 3rd ed. Moulton JE, eds. University of California Press, California. 231–301, 1990
20) Mutz M, Boudreaux B, Kearney M, Strouda K, Gaunt S, Shiomitsu K. Prognostic value of baseline absolute lymphocyte concentration and neutrophil / lymphocyte ratio in dogs with newly diagnosed multicentric lymphoma. Vet Comp Oncol 13, 337–347, 2015
21) Nakamura N, Momoi Y, Watari T, Yoshino T, Tsujimoto H, Hasegawa A. Plasma thymidine kinase activity in dogs with lymphoma and leukemia. J Vet Med Sci 59, 957–960, 1997
22) Pavel C, Manolescu N. Haematological modifications in some malignant tumours of companion animals. Lucrări Ştiintifice Medicină Veterinară 40, 280–292, 2007
23) Priester WA, McKay FW. The occurrence of tumors in domestic animals. Natl Cancer Inst Monogr 54, 1–210, 1980
24) Sapierzyński R, Micuń J. Lymphadenomegaly in dogs-cytological study. Pol J Vet Sci 12, 263–268, 2009
25) Sapierzyński R, Micuń J, Jagielski D, Jurka P. Cytopathology of canine lymphomas (100 cases). Pol J Vet Sci 13, 653–659, 2010
26) Sharif H, von Euler H, Westberg S, He E, Wang L, Eriksson S. A sensitive and kinetically defined radiochemical assay for canine and human serum thymidine kinase 1 (TK1) to monitor canine malignant lymphoma. Vet J 194, 40–47, 2012
27) Sozmen M, Tasca S, Carli E, De Lorenzi D, Furlanello T, Caldin M. Use of fine needle aspirates and flow cytometry for the diagnosis, classification, and immunophenotyping of canine lymphomas. J Vet Diagn Invest 17, 323–329, 2005
28) Teske E. Canine malignant lymphoma: A review and comparison with human non Hogkins lymphoma. Vet Q 4, 209–219, 1994
29) Teske E, van Heerde P, Rutteman GR, Kurzman I, Moore PF, MacEwen EG. Prognostic factors for treatment of malignant lymphoma in dogs. J Am Vet Med Assoc 205, 1722–1728, 1994
30) Teske E, van Heerde P. Diagnostic value and reproducibility of fine-needle aspiration cytology in canine malignant lymhpoma. Vet Q 18, 112–115, 1996
31) Van Pelt RW, Connor GH. Clinicopathologic survey of malignant lymphoma in the dog. J Am Vet Med Assoc 152, 976–989, 1968
32) Vezzali E, Parodi AL, Marcato PS, Bettini G. Histopathologic classification of 171 cases of canine and feline non-Hodgkin lymphoma according to the WHO. Vet Comp Oncol 8, 38–49, 2010
33) von Euler H, Einarsson R, Olsson U, Lagerstedt AS, Eriksson S. Serum thymidine kinase activity in dogs with malignant lymphoma: a potent marker for prognosis and monitoring the disease. J Vet Intern Med 18, 696–702, 2004
34) Williams LE, Johnson JL, Hauck ML, Ruslander DM, Price GS, Thrall DE. Chemotherapy followed by half-body radiation therapy for canine lymphoma. J Vet Intern Med 18, 703–739, 2004
35) Zanatta R, Abate O, D'Angelo A, Miniscalco B, Mannelli A. Diagnostic and prognostic value of serum lactate dehydrogenase (LDH) and LDH isoenzymes in canine lymphoma. Vet Res Commun 27, 449–452, 2003
36) Zandvliet M. Canine lymphoma: a review. Vet Q 36, 76–104, 2016