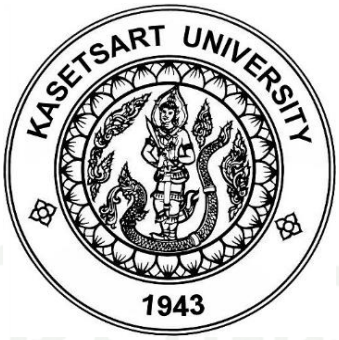

THESIS

EFFECTIVENESS AND ADVERSE EVENTS OF COP CHEMOTHERAPY IN FELINE MEDIASTINAL LYMPHOMA NATURALLY INFECTED WITH FELINE LEUKEMIA VIRUS

SUPITA SUNPONGSRI

GRADUATE SCHOOL, KASETSART UNIVERSITY
Academic Year 2021

THESIS APPROVAL
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DEGREE: Master of Science (Veterinary Clinical Studies)
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TITLE: Effectiveness and Adverse Events of COP Chemotherapy in Feline Mediastinal Lymphoma Naturally Infected with Feline Leukemia Virus

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THESIS

EFFECTIVENESS AND ADVERSE EVENTS OF COP CHEMOTHERAPY IN FELINE MEDIASTINAL LYMPHOMA NATURALLY INFECTED WITH FELINE LEUKEMIA VIRUS

SUPITA SUNPONGSRI

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Veterinary Clinical Studies)
Graduate School, Kasetsart University
Academic Year 2021

Supita Sunpongsri : Effectiveness and Adverse Events of COP Chemotherapy in Feline Mediastinal Lymphoma Naturally Infected with Feline Leukemia Virus. Master of Science (Veterinary Clinical Studies), Major Field: Veterinary Clinical Studies, Faculty of Veterinary Medicine.
Thesis Advisor: Assistant Professor Tassanee Jaroensong, Ph.D.
Academic Year 2021

Feline leukemia virus (FeLV) infection is considered a poor prognostic factor for feline lymphoma. This study investigated the prevalence of cats suffering from feline lymphoma with natural infection of the feline leukemia virus, as well as clinical signs, adverse events, and survival time after cyclophosphamide, vincristine, and prednisolone (COP) chemotherapy. This retrospective study involved 92 cats, diagnosed with mediastinal or mediastinal plus other anatomical sites of lymphoma and treated with COP chemotherapy. FeLV-antigen-positive was observed in all cats. Clinical signs and adverse events were observed after the 1st, 2nd, and 3rd inductions. Clinical signs improved after the 3rd induction of COP chemotherapy. The response rate was 96.74% (81.52% complete response, 15.22% partial response, and 3.26% no response). The overall median survival time was 338 days (range 62–1,057 days). The overall response rate and median survival time of feline lymphoma that were FeLV-antigen-positive and treated with COP chemotherapy were higher than from other studies. This study found that cats aged <4 years survived longer than those aged at least 4 years. Anemia (before COP), azotemia (after 2nd induction), and elevated alanine aminotransferase (after 1st induction) were associated with an increased chance of mortality.

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Student's signature Thesis Advisor's signature

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Last, but not least, my warm and heartfelt thanks go to my family for all the strength they have given me. I love you all.

Supita Sunpongsri

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INTRODUCTION

Lymphoma is one of the most common tumors leading to deaths in both humans and companion animals worldwide (1, 2). Lymphoma represents 83% of canine (3) and 90% of feline (4) hematopoietic-origin tumors.

Various associations have been reported between lymphoma, age, sex, neutering status, and breed (5-11). Lymphoma can be classified by anatomical types into multicentric, mediastinal, renal, hepatic, nodal, and alimentary (12) that can be characterized as solitary, diffuse, or multifocal (13).

Progressive FeLV-infected cats have around a 60-fold greater risk of developing lymphoma compared with non-infected cats. Mediastinal lymphoma (especially in the prevaccination era in high prevalence areas) is the most common form of FeLV-associated lymphoma, followed by multicentric lymphoma (11, 14, 15).

Clinical signs from asymptomatic to severe vary for cats with retroviral infection and lymphoma. Severe clinical signs can result in acute death (16). Respiratory distress from pleural effusion is usually associated with feline mediastinal lymphoma (17).

There are many chemotherapy protocols available for the treatment of feline lymphoma (4, 7, 8, 18-21). Complete and partial response rates as well as median survival rate were reported to be not significantly different between the two protocols: COP (cyclophosphamide, vincristine and prednisolone) and Madison-Wisconsin (7).

The adverse events of chemotherapy usually occurred within 24–48 hours or with acute delay within 2–14 days after treatment (19, 22). To our knowledge, the association between adverse events and survival time has not been reported.

Investigation of the onset and duration of clinical signs and adverse events from COP chemotherapy may benefit feline lymphoma treatments and prognosis.

OBJECTIVES

1. To identify the prevalence of cats naturally infected with FeLV in feline lymphoma.

2. To investigate the clinical signs, adverse events, and survival time of COP chemotherapy in feline lymphoma.

LITERATURE REVIEW

Lymphoma is one of the most common tumors that leading to deaths in both humans and companion animals worldwide (1). According to American Cancer Society's estimates for non-Hodgkin's lymphoma in 2021 are accounting for about 4% of all cancers. Lymphoma represented 83% of canine (3) and 90% of feline (4) hematopoietic origin tumors.

Lymphoma can be classified by anatomical types into multicentric (43.4%), mediastinal (33.96%), renal (11.32%), hepatic (5.66%), nodal (3.77%) and alimentary (1.89%) (12) which characterized by solitary, diffuse, or multifocal (13).

Lymphoma in cats presented in older cats with a median age >7 years in cats with negative feline leukemia virus (FeLV) (5, 6). Other studies of mediastinal lymphoma in cats were reported a median age as 3 years or bimodal age distribution (8, 9). Male cats are predisposing in some studies (5, 9, 10) while other studies have no association between lymphoma, sex and neutering status (6, 11). Several studies reported that Siamese breeds were over-represented in cats with lymphoma (7, 9, 11). Progressive FeLV-infected cats have around 60-fold risk of developing lymphoma compared with non-infected cats.

Feline leukemia virus is a retrovirus that belongs to the oncornavirus subfamily of gammaretroviruses. FeLV is a single-stranded RNA with reverse transcriptase, integrase, and protease packaged into a capsid protein p27. It replicates within bone marrow, salivary glands, and respiratory epithelium. It's integrated into the host genomic DNA, called proviral DNA. FeLV is primarily transmitted through saliva. Horizontal transmission among cats were close contact, fighting and biting, fleas and flea feces, and blood transfusions. Transplacental transmission occurs from queen to kitten (14).

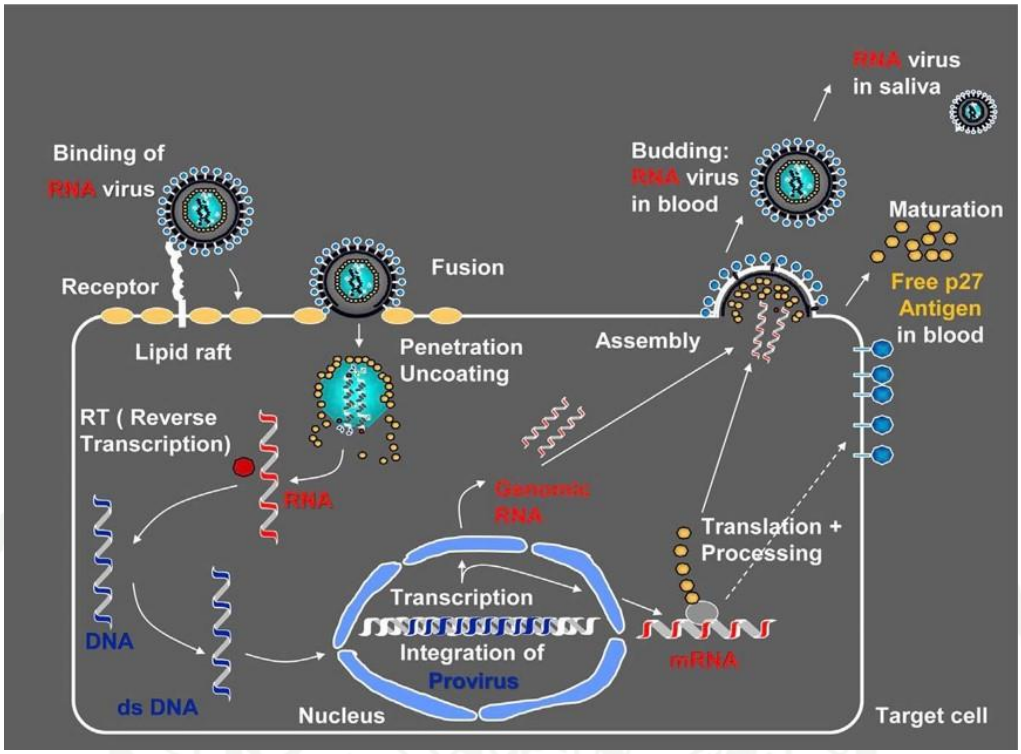

Figure 1 FeLV replication.

FeLV infection is found in domestic cats all over the world. Prevalence of progressive FeLV infection was 2.3% to 3.3% in the United States, 0.7% to 15.6% in Europe, 3.0% to 28.4% in South America, and 0.5% to 24.5% in Asia and Australia/New Zealand (14, 23). FeLV prevalence is higher in intact males, aggressive cats (fights, bites), cats with social behavior of grooming and sharing food bowls, and multicat household. FeLV prevalence in sick cats was as high as 38%.

FeLV infected cats can develop degenerative diseases, myelosuppression or opportunistic infections including hematopoietic neoplasms, mainly lymphoma (24). Mediastinal lymphoma (especially prevaccination era) is the most common form of FeLV- associated lymphoma followed by multicentric lymphoma (11, 14, 15).

FeLV infected cats cause lymphoma is through insertion of a provirus near a cellular oncogene (most commonly *myc*) in the host's genome. The integrated FeLV provirus can interrupt or inactivate cellular genes in the infected cells resulting in activation and overexpression of that gene. These effects cause the cells to proliferate uncontrollably (14, 25-27).

Clinical signs of cats with retroviral infection (as Shown in Table 1) and lymphoma (including polyuria/polydipsia, oral lesion, digestive disorders, neurologic disorders, respiratory disorders, and anemia) are varies from asymptomatic to severe which can lead to acute death (16).

Table 1 Clinical signs associated with feline retroviral infection.

Clinicopathological sign	**Score**		
	0	**1**	**2**
Loss of appetite	No	Partial (disorexia)	Total (anorexia)
Asthenia	No	Slightly depression	Severe-prostration
Dehydration	No	< 10%	> 10%
Weight loss	No	Thinness	Cachexia
Lymphadenomegaly	No	Localized	Generalized
Altered mucosae	No	Pale-congestive	
Polyuria/Polydipsia	No	Yes	
Conjunctivitis	No		Yes
Keratitis	No		Yes
Oral lesions	No		Yes
Digestive disorders	No	Yes	
Cutaneous lesions	No	Pruritus, alopecia	Nodules, ulcers
Respiratory disorders	No	Mild	Severe
Neurologic disorders	No		Yes
Lymphoma	No		Yes
Myeloproliferative disorders	No		Yes
Other neoplasia	No		Yes

Pleural effusion in cats was usually caused by cardiac disease (35.3%), neoplasia (30.7%), pyothorax (8.8%), feline infectious peritonitis (8.5%), chylothorax (4.6%). Mediastinal lymphoma is a neoplasia that usually found in cats with respiratory distress (17).

There are many chemotherapy protocols available for treatment of feline lymphoma (4, 7, 8, 18-20, 22). Lymphoma has high response rate to chemotherapy with a complete response (CR) >60% (7, 8, 10, 18, 19, 21, 28). Complete (CR) and partial response (PR) rates as well as median survival rate were not significantly between protocols: COP (cyclophosphamide, vincristine, and prednisolone) and Madison–Wisconsin (MW) (7).

COP protocol (according to Teske (8)), combination of 3 drugs, vincristine 0.5-0.75 mg/m^2 IV (day 1, 8, 15, 22), cyclophosphamide 300 mg/m^2 PO (day 1, 22) and prednisone 1-2 mg/kg PO daily. After day 22, vincristine and cyclophosphamide given every 3 weeks until relapse or for one year.

Cyclophosphamide active metabolites covalently bind alkyl groups to DNA to form bifunctional adducts that generate interstrand and/or intrastrand breaks and disruption of DNA synthesis and subsequent cell death. Vincristine binds to distinct site on tubulin proteins to inhibit tubulin polymerization and microtubule assembly and disruption of the mitotic spindle, metaphase arrest and subsequent cell death. Prednisolone binds to specific cytoplasmic receptors on cancer lymphocytes in nucleus and also alters DNA synthesis leads to apoptosis (29, 30).

Lymphoma treated with chemotherapy has overall median survival time (MST) of 97 to 484 days (4, 7, 8, 10, 18, 19, 21, 28). Cats with FeLV-infected has poor prognosis with MST from 37 to 126.3 days and disease-free interval (DFI) from 27 to 71 days (4, 10, 18).

The common adverse events of chemotherapy usually occurred immediately within 24-48 hours or acute delay within 2-14 days after treatment. Anorexia, vomiting and hematologic toxicity (anemia, neutropenia), most of them were associated with cyclophosphamide (19, 22). Severity of adverse events (as shown in Table 2) were graded according to the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) as grade I-V toxicity (31).

Table 2 Common terminology criteria for adverse events following chemotherapy in cats

Adverse event	Grade				
	I	**II**	**III**	**IV**	**V**
Packed cell volume	25%-<LLN	20-<25%	15-<20%	<15%	Death
Neutropenia	1500/µL-<LLN	1000-1499/µL	500-999/µL	<500/µL	Death
Creatinine	>1.0-1.5× baseline	>1.5-3.0× baseline	>3.0× baseline	>3.0x ULN	-
Alanine aminotransferase	>ULN to 1.25× ULN	>1.25-1.5× ULN	>1.5-2.0× ULN	>2.0× ULN	-
Anorexia	Coaxing or dietary change required to Maintain appetite	Oral intake altered (≤3 days) without significant weight loss; oral nutritional supplements/appetite stimulants may be indicated	Of >3 days duration; associated with significant weight loss (≥10%) or malnutrition; IV fluids, tube feeding or force feeding indicated	Life-threatening consequences; TPN indicated; >5 days duration	Death
Vomit	<3 episode in 24 h, medical intervention not indicated	3-10 episodes in 24 h; <5 episodes/day for ≤48 h; parenteral fluids (IV or SC) indicated ≤48 h; medications indicated	Multiple episodes >48 h and IV fluids or PPN/TPN indicated >48 h	Life-threatening (e.g.haemodynamic collapse)	Death
Diarrhea	Increase of up to 2 stools per day over baseline; no increase in frequency, however, consistency decreased	Increase of 3-6 stools per day over baseline; medications indicated; parenteral (IV or SC) fluids indicated ≤48 h; not interfering with ADL	Increase of >6 stools per day over baseline; incontinence >48 h; IV fluids >48 h; hospitalization; interfering with ADL	Life-threatening (e.g.haemodynamic collapse)	Death

LLN, lower limit of normal; ULN, upper limit of normal; IV, intravenous; SC, subcutaneous; PPN, partial parenteral nutrition; TPN total parenteral nutrition; ADL = activities of daily living (eating, sleeping, defecating and urinating).

MATERIALS AND METHODS

Clinical evaluation and treatment

This research involved a retrospective study that considered the medical records of client-owned cats diagnosed with mediastinal lymphoma at the Kasetsart University Veterinary Teaching Hospital (Bangkok, Thailand). The database was compiled by reviewing the cases of hospital visits between June 2019 and June 2020.

Various data on the treated cats were recorded: age, sex, breed, reproductive status, clinical signs (Table 3; modified according to Collado (16)), retroviral status, adverse events of chemotherapy (Table 4; modified according to VCOG-CTCAE v2 (31)), response to chemotherapy, remission, and survival time. The clinical signs observed and evaluated in cats to obtain a clinical score (CS) from the day of diagnosis to after 3rd induction. CS were classified into 3 clinical groups (CG): CG1, with no clinical signs (asymptomatic); CG2, with CS 1-5 (mild disease); and CG3, with CS ≥ 6 (severe disease). The anatomical location of each lymphoma was divided into mediastinal and mediastinal plus other sites.

Table 3 Clinical signs of feline lymphoma with FeLV infected.

Clinicopathological sign	**Score**		
	0	**1**	**2**
Respiratory disorders	No	Mild	Severe
Loss of appetite	No	Partial	Total
Lymphadenomegaly	No	Localized	Generalized
Asthenia postration	No	Slightly	Severe
Dehydration	No	< 10%	> 10%
Weight loss	No	Thinness	Cachexia
Oral lesions	No	Mild	Severe
Neurologic disorders	No	Ataxia	Seizure
Conjunctivitis	No	Yes	
Skin lesion	No	Yes	
Pale mucous membrane	No	Yes	
Polyuria/Polydipsia	No	Yes	

Table 4 Adverse events grading of COP chemotherapy in cats.

Adverse event	Grade				
	I	**II**	**III**	**IV**	**V**
Anemia (%)	25-29	20-24.9	15-19.9	<15	Death
Leukopenia (x10^3/cumm)	3.0-5.0	1-2.99	0.5-0.99	<0.5	Death
Neutropenia (x10^3/cumm)	1,500-2,500	1,000-1,499	500-999	<500	Death
sCr (mg%)	1.61-2.2	2.21-2.8	2.81-5.0	>5.0	Death
ALT (37°C IU/L)	140-280	281-350	351-420	>420	Death
Vomit	<3 episode in 24 h	3-10 episodes in 24 h; parenteral fluids (IV or SC) indicated ≤48 h; medications indicated	Multiple episodes >48 h and IV fluids or PPN/TPN indicated >48 h	Life-threatening	Death
Diarrhea	Fecal score 4/7	Fecal score 5-6/7; parenteral (IV or SC) fluids indicated ≤48 h	Fecal score 7/7; IV fluids >48 h; hospitalization	Life-threatening	Death
Anorexia	Dietary change required to Maintain appetite	Oral intake altered (≤3 days) without significant weight loss	Significant weight loss (≥10%); IV fluids, tube feeding or force feeding indicated	Life-threatening	Death

COP, cyclophosphamide, vincristine, and prednisolone; IV, intravenous; SC, subcutaneous; PPN, partial parenteral nutrition; TPN total parenteral nutrition.

Inclusion criteria were a cytological or histopathological diagnosis of lymphoma from pleural effusion or cranial mediastinal mass and the presence of a cranial mediastinal mass from thoracic radiography or ultrasonography.

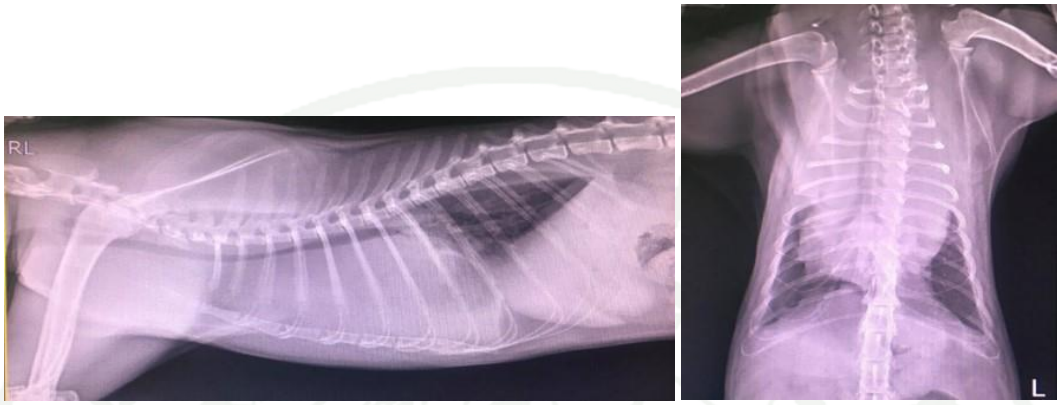

Figure 2 Thoracic radiography
Right lateral and ventrodorsal thoracic radiograph with cranial mediastinal mass

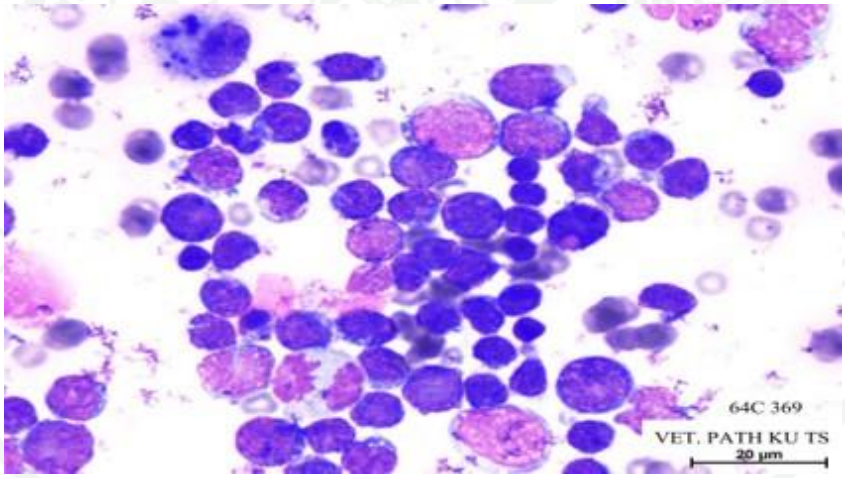

Figure 3 Lymphoma cytology
Round cells characterized by moderately abundant basophilic cytoplasm and eccentric oval shape nucleus. These cells were markedly anisocytosis and anisokaryosis. These cells were approximately 1.5-4 times of Rbc's size.

FeLV testing was performed using rapid immune-migration (RIM)-based methods (WITNESS®). Cats were negative using the test kit FeLV antigen had a whole blood nested polymerase chain reaction (PCR) analysis for FeLV provirus, as shown in Table 5 (32).

Table 5 Nested polymerase chain reaction (PCR) analysis for FeLV provirus.

Primer	Region	Sequence (5'→3')	Product size (bp)
U3-F(1)	U3 region	ACA GCA GAA GTT TCA AGG CC	770
G-R(1)	*gag* gene	GAC CAG TGA TCA AGG GTG AG	
U3-F(2)	U3 region	GCT CCC CAG TTG ACC AGA GT	601
G-R(2)	*gag* gene	GCT TCG GTA CCA AAC CGA AA	

All cats were treated using the COP protocol (according to Teske (8)), combination of 3 drugs, vincristine 0.5-0.75 mg/m^2 IV (day 1, 8, 15, 22), cyclophosphamide 300 mg/m^2 PO (day 1, 22) and prednisone 1-2 mg/kg PO daily. After day 22, vincristine and cyclophosphamide given every 3 weeks until relapse or for one year.

During COP chemotherapy if leukopenia (<3,000/μL) or neutropenia (<1,500/μL) occurred, the COP chemotherapy was delayed. In cats with leukopenia or neutropenia LEUKOPLUS® (a white blood cell growth factor analogue) at a dose of 3-10 μg/kg was administered subcutaneously for three consecutive days. If the adverse events progressed to grade IV, the chemotherapy was terminated and changed to palliative treatment. Cats were excluded if they had been treated with a chemotherapy protocol other than the COP protocol as the first line or if medical records were incomplete.

Statistical analysis

Factors analyzed for response to COP chemotherapy were breed, age, sex, retroviral status, clinical signs, and adverse events. The Kaplan-Meier log rank test and the Cox proportional hazard method were used to estimate survival analysis for which the survival time was calculated from the date of diagnosis to the date of death. Cats were censored if they were alive at the end of the study or if lost to follow-up. A P-value < 0.05 was considered statistically significant. All statistical analyses were performed using the R software (R Core Team, 2020) in the RStudio Desktop 1.3.1093 environment with the Rcmdr, Survival and Survminer packages.

RESULTS

Between June 2019 and June 2020, 122 cats that had presented for lymphoma and been treated with COP chemotherapy were reviewed. In total, 92 cats met the mediastinal lymphoma inclusion criteria.

The median age was 2 years (range 8 months to 11 years). There were 41 neutered males (44.57%), 19 intact males (20.65%), 15 spayed females (16.30%), 11 intact females (11.96%), 2 unknown males, and 4 unknown females. The breeds represented were: 88 domestic shorthairs (95.65%), 2 Persians, and 2 Scottish folds.

All 92 cats had FeLV-antigen-positive. Tumor anatomical location was mainly recorded only as mediastinal for 78 cats (84.78%) and as mediastinal plus other sites for 14 cats (15.22%).

The number of cats presenting with dyspnea and requiring thoracocentesis before COP chemotherapy was 65 (70.65%). After induction first COP chemotherapy, there were 23 cats (25%) that had one or more thoracocentesis performed, while 69 cats (75%) no longer required thoracocentesis. The descriptive data of cats recruited in the study were summarized in Table 6.

As shown in Table 7, the most common clinical signs on the day of diagnosis were: respiratory disorders (71.74%), loss of appetite (38.05%), lymphadenomegaly (18.86%), asthenia (25%), dehydration (21.74%), weight loss (14.13%), oral lesion (11.54%), pale mucous membranes (4.35%), neurologic disorders (3.26%), and conjunctivitis (1.09%).

After the 1st induction, common clinical signs were loss of appetite and weight loss. All clinical signs, including conjunctivitis, skin lesions and diarrhea, diminished following the 3rd induction of COP chemotherapy.

According to the current VCOG-CTCAE grading system (31), the adverse effects of COP chemotherapy were hematologic toxicity (anemia, leukopenia, neutropenia, azotemia and elevated alanine aminotransferase (ALT)) and signs of anorexia, vomit, or diarrhea, as indicated in Table 8.

Anemia is the most common COP chemotherapy adverse event, followed by leukopenia, azotemia, elevated ALT, and neutropenia. The common adverse events signs were anorexia, vomit, and diarrhea, respectively. The majority of adverse events

were grade I except for 1 cat who had anemia grade IV and 4 cats who had elevated ALT grade IV after the first induction.

Table 6 Patient characteristics.

Variable	**n**	**%**
Age (yr)		
<1	8	8.70
1-2	35	38.04
2-3	20	21.74
3-4	15	16.30
>4	14	15.22
Sex		
Male	62	67.39
Female	30	32.61
Breed		
Domestic Shorthairs	88	95.65
Others (Scottish fold, Persian)	4	4.35
Status		
Neutered	56	60.87
Intact	30	32.61
Unknown	6	6.52
Anatomical location		
Mediastinal	78	84.78
Mediastinal + others sites	14	15.22
Thoracocentesis before COP		
Yes	65	70.65
No	27	29.35
Thoracocentesis after COP (times)		
0	69	75.00
1	16	17.39
>1	7	7.61
Blood transfusion before 4^{th} week		
Yes	6	6.52
No	86	93.48
Blood transfusion after 4^{th} week		
Yes	10	10.87
No	82	89.13
COP response		
Complete	75	81.52
Partial	14	15.22
No	3	3.26

COP, cyclophosphamide, vincristine, and prednisolone.

Table 7 Clinical signs of feline lymphoma on day of diagnosis, after 1st, 2nd, and 3rd induction of COP chemotherapy.

Clinicopathological sign		Before COP		After 1st induction		After 2nd induction		After 3rd induction	
		No. cats	Percentage	No. cats	Percentage	No. cats	Percentage	No. cats	Percentage
Respiratory disorders	Score 1	54/92	58.70	14/92	15.22	0/92	0.00	1/91	1.10
	Score 2	12/92	13.04	0/92	0.00	1/92	1.09	0/91	0.00
Loss of appetite	Score 1	27/92	29.35	19/92	20.65	12/92	13.04	4/91	4.40
	Score 2	8/92	8.70	1/92	1.09	1/92	1.09	0/91	0.00
Lymphadenomegaly	Score 1	5/53	9.43	5/66	7.58	3/65	4.62	0/71	0.00
	Score 2	5/53	9.43	3/66	4.55	3/65	4.62	0/71	0.00
Asthenia postration	Score 1	19/92	20.65	5/92	5.43	3/92	3.26	1/91	1.10
	Score 2	4/92	4.35	0/92	0.00	0/92	0.00	0/91	0.00
Dehydration	Score 1	20/92	21.74	8/92	8.70	7/92	1.00	1/91	1.10
Weight loss	Score 1	9/92	9.78	22/92	23.91	11/92	11.96	7/91	7.69
	Score 2	4/92	4.35	2/92	2.17	1/92	1.09	1/91	1.10
Oral lesions	Score 1	2/43	4.65	0/42	0.00	0/41	0.00	0/43	0.00
	Score 2	3/43	6.98	3/42	7.14	1/41	2.44	0/43	0.00
Neurologic disorders	Score 1	1/92	1.09	0/92	0.00	0/92	0.00	0/91	0.00
	Score 2	2/92	2.17	1/92	1.09	1/92	1.09	1/91	1.10
Conjunctivitis		1/92	1.09	0/92	0.00	1/92	1.09	0/91	0.00
Skin lesion		0/92	0.00	1/92	1.09	0/92	0.00	0/91	0.00
Pale mucous membranes		4/92	4.35	6/92	6.52	5/92	5.43	4/91	4.40
Polyuria/Polydipsia		0/92	0.00	0/92	0.00	0/92	0.00	0/91	0.00

COP, cyclophosphamide, vincristine, and prednisolone.

Table 8 Adverse events related to COP chemotherapy in cats.

Adverse event		Before COP		After 1st induction		After 2nd induction		After 3rd induction	
		No. cats	Percentage	No. cats	Percentage	No. cats	Percentage	No. cats	Percentage
Anemia	Grade I	7/92	7.61	25/92	27.17	23/91	25.27	15/90	16.67
	Grade II	4/92	4.35	9/92	9.78	14/91	15.38	16/90	17.78
	Grade III	1/92	1.09	4/92	4.35	2/91	2.20	1/90	1.11
	Grade IV	0/92	0.00	1/92	1.09	1/91	1.10	0/90	0.00
Leukopenia	Grade I	1/92	1.09	12/92	13.04	18/91	19.78	7/90	7.78
	Grade II	0/92	0.00	2/92	2.17	1/91	1.10	1/90	1.11
Neutropenia	Grade I	0/89	0.00	3/86	3.49	5/89	5.62	5/88	5.68
	Grade II	0/89	0.00	1/86	1.16	2/89	2.25	1/88	1.14
	Grade III	0/89	0.00	0/86	0.00	1/89	1.12	0/88	0.00
sCr	Grade I	28/86	32.56	14/88	15.91	10/84	11.90	14/88	15.91
	Grade II	2/86	2.33	0/88	0.00	0/84	0.00	1/88	1.14
	Grade III	1/86	1.16	0/88	0.00	0/84	0.00	0/88	0.00
ALT	Grade I	1/86	1.16	4/86	4.65	1/81	1.23	3/88	3.41
	Grade II	0/86	0.00	0/86	0.00	1/81	1.23	0/88	0.00
	Grade III	0/86	0.00	0/86	0.00	0/81	0.00	2/88	2.27
	Grade IV	3/86	3.49	4/86	4.65	3/81	3.70	3/88	3.41
Anorexia	Grade I	22/92	23.91	15/92	16.30	9/92	9.78	4/91	4.40
	Grade II	7/92	7.61	6/92	6.52	3/92	3.26	1/91	1.10
	Grade III	6/92	6.52	3/92	3.26	2/92	2.17	1/91	1.10
Vomiting	Grade I	7/92	7.61	5/92	5.43	6/92	6.52	4/91	4.40
	Grade II	6/92	6.52	0/92	0.00	0/92	0.00	1/91	1.10
Diarrhea	Grade I	0/92	0.00	1/92	1.09	2/92	2.17	4/91	4.40

COP, cyclophosphamide, vincristine, and prednisolone; sCr, serum creatinine; ALT, alanine aminotransferase.

Based on the survival analysis of 76 cats, the overall response rate was 96.74% (81.52% complete response, 15.22% partial response, and 3.26% no response). At the end of the study, 52 cats (68.42%) had died and 24 cats (31.58%) were alive (median: 518 days, range 336–1,057 days). The overall MST of the 76 cats was 338 days (range 62–1,057 days; Figure 4). Cats with a complete response (MST 379 days) had significantly ($p = 0.008$) longer survival times than those with a partial response (MST 134 days) and no response (MST 121 days), as shown in Figure 5. In total, 81 cats had a mean disease-free interval of 242 days (range 12–1,017 days).

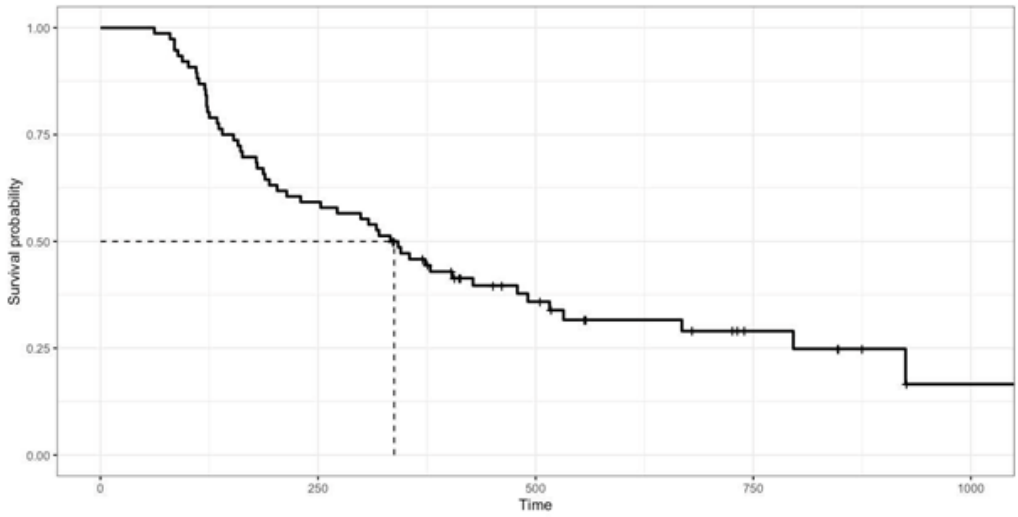

Figure 4 Kaplan-Meier survival curve showing overall median survival.
Median survival time of 76 cats with mediastinal lymphoma treated with COP chemotherapy was 338 days (range 62–1,057 days).

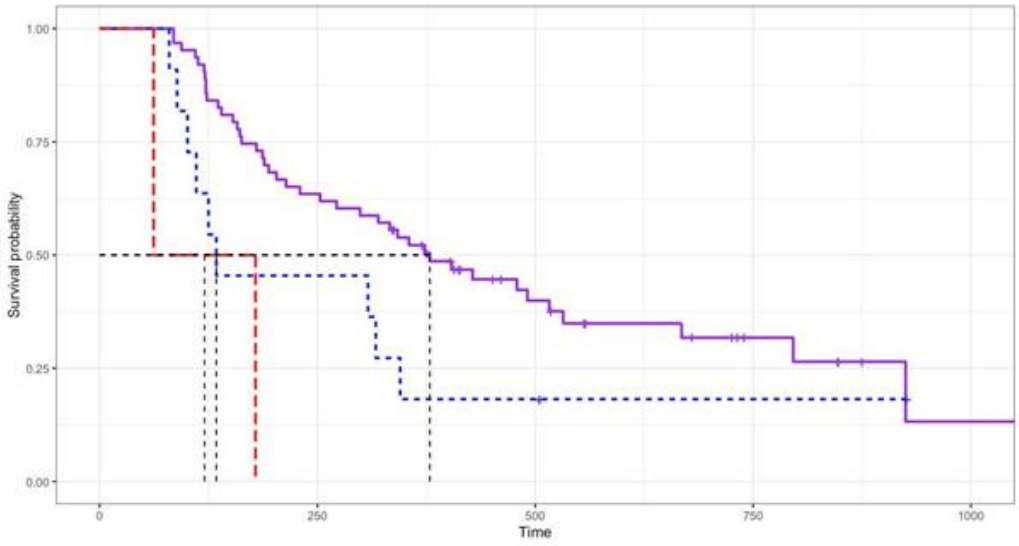

Figure 5 Log rank test analysis showing response to COP chemotherapy.
Cats with complete response (MST 379 days; purple line) had highly significantly (p = 0.008) longer survival times than those with partial response (MST 134 days; blue line) and no response (MST 121 days; red line).

The median survival times based on sex, reproductive status, retroviral status, anatomical location, and blood transfusion after COP were not different, according to the log rank test analysis of the 76 cats. Cats aged <4 years (MST 373 days) had longer survival times than those aged at least 4 years (MST 212 days) ($p = 0.01$; Figure 6). After 1st induction of COP, cats that required thoracocentesis (MST 147 days) had shorter survival times ($p = 0.002$) than those not requiring thoracocentesis (MST 373 days; Figure 7).

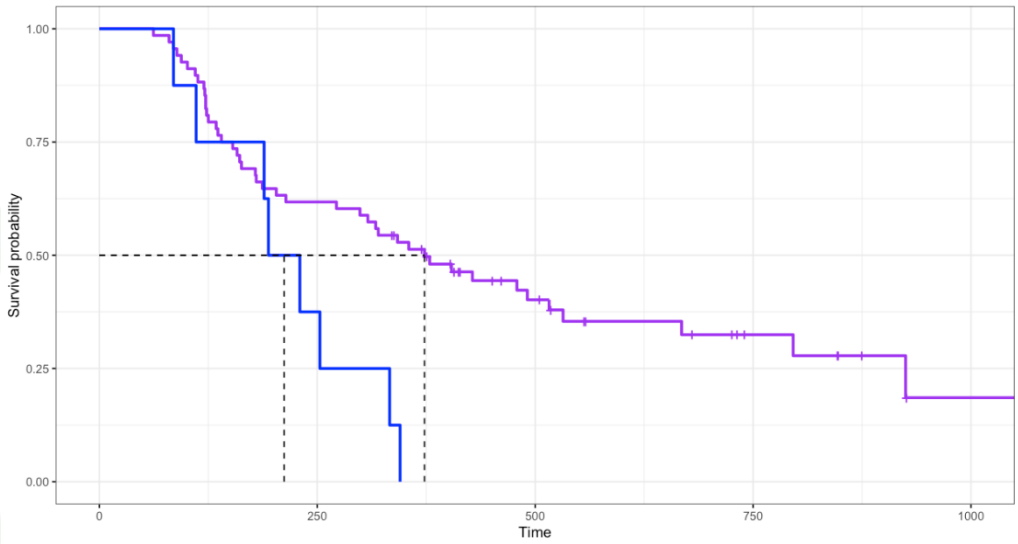

Figure 6 Log rank test analysis showing aged-group of cats.
Cats aged <4 years (MST 373 days; purple line) had longer survival times (p = 0.01) than those aged at least 4 years (MST 212 days; blue line).

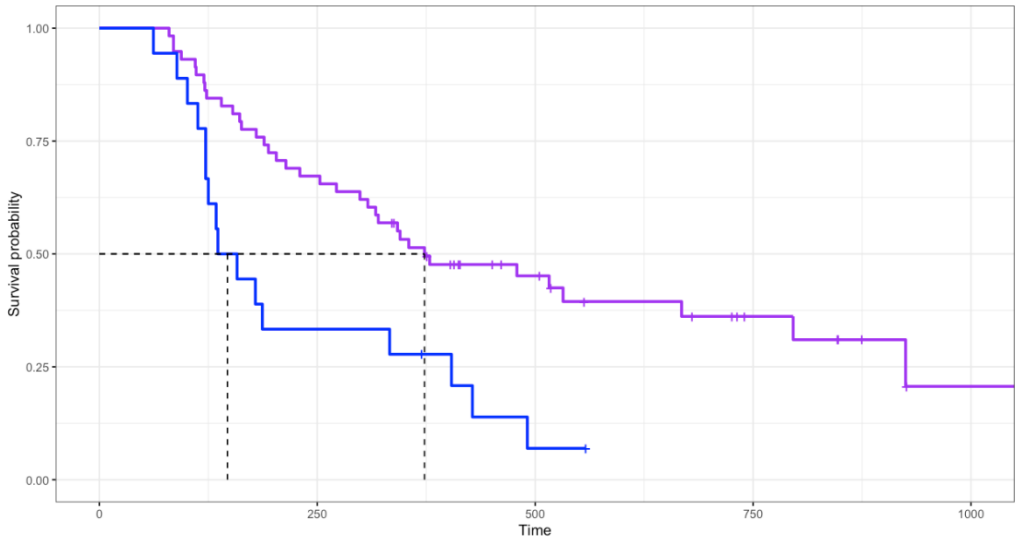

Figure 7 Log rank test analysis showing thoracocentesis after 1st induction.
Cats that required thoracocentesis after 1st induction (MST 147 days) had shorter survival times (p = 0.002) than those not requiring thoracocentesis (MST 373 days).

Table 9 summarizes the results of the Cox-regression analysis of the 76 cats. Cats with anemia (before COP), azotemia (after 2nd induction) and elevated ALT (after 1st induction) had an increased hazard of death. Through multivariable analysis, cats with anemia before COP (p = 0.013; hazard ratio (HR) = 2.5; Figure 8), with azotemia after 2nd induction (p = 0.044; HR = 2.3; Figure 9), with elevated ALT after 1st induction (p = 0.014; HR = 2.8; Figure 10), all had a higher risk of death.

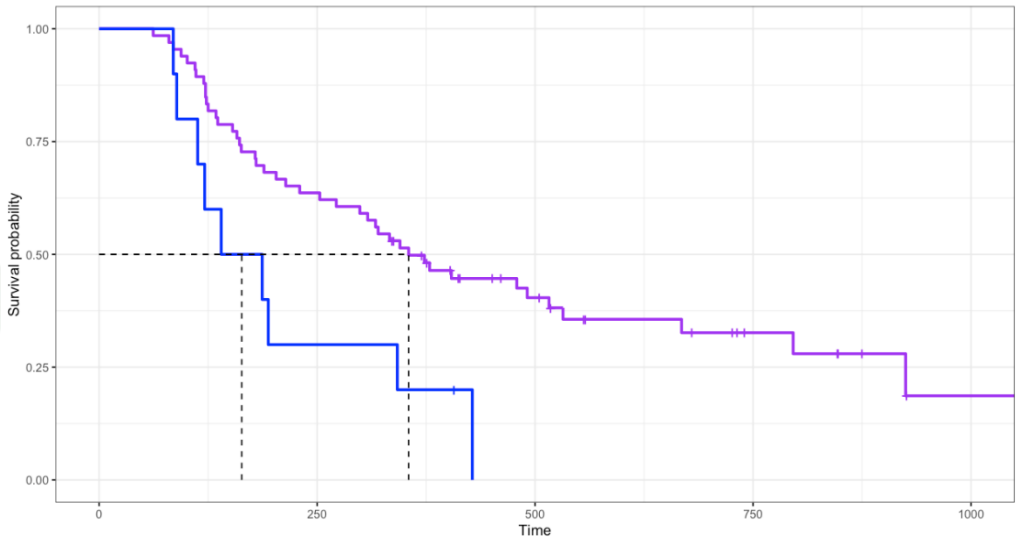

Figure 8 Log rank test analysis showing anemic cats before COP chemotherapy. Cats with anemia before COP (MST 164 days) had shorter survival times (p = 0.01) than those with normal packed cell volume (MST 355 days).

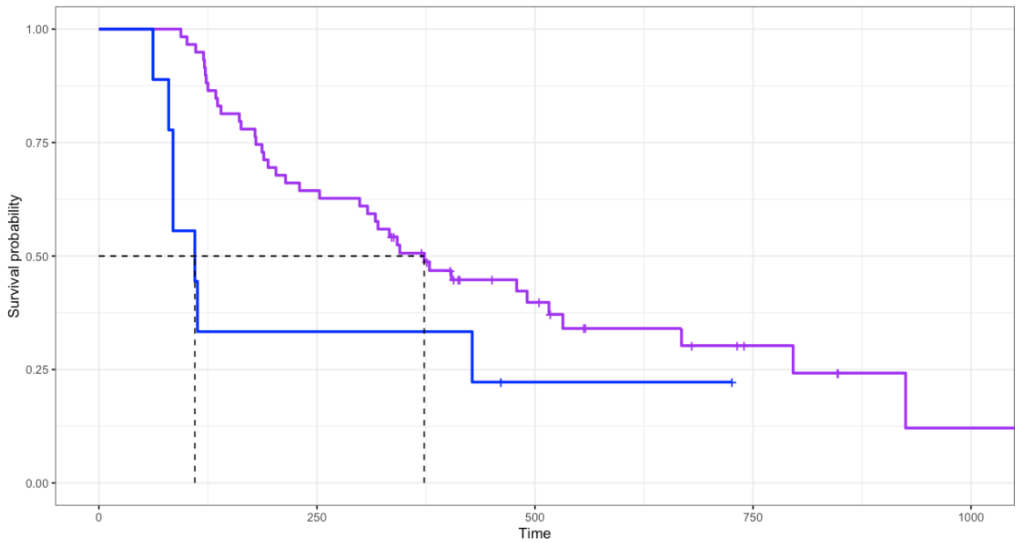

Figure 9 Log rank test analysis showing azotemia cats after COP chemotherapy. Cats with azotemia after 2nd induction (MST 110 days) had shorter survival times (p = 0.04) than those with normal serum Creatinine (MST 373 days).

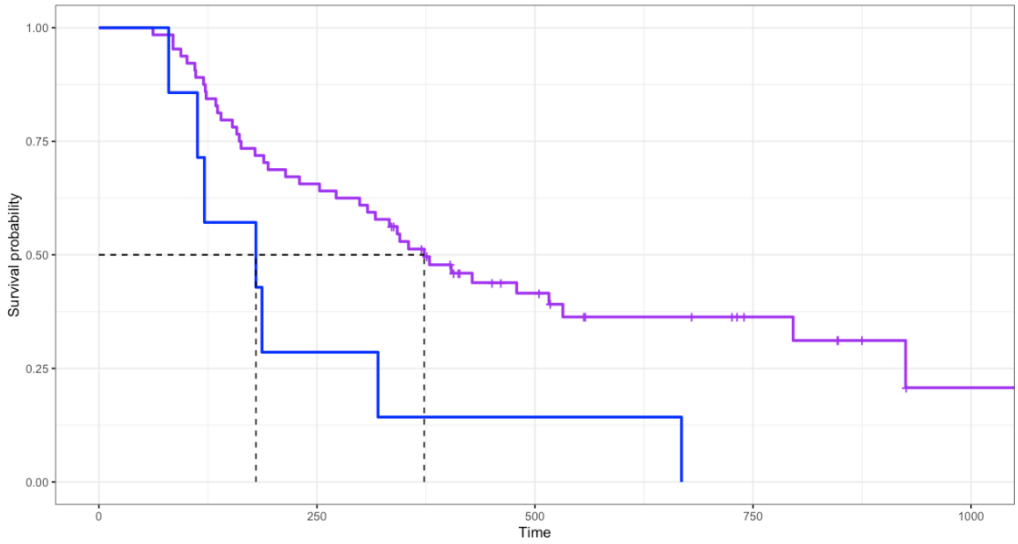

Figure 10 Log rank test analysis showing elevated ALT cats after COP chemotherapy. Cats with elevated ALT after 1st induction (MST 180 days) had shorter survival times (p = 0.01) than those with normal ALT (MST 373 days).

Table 9 Cox-regression analysis of 76 cats treated with COP chemotherapy.

Factor	**Hazard ratio**	**95% CI**	***p***
Age	2.6	1.2-5.7	0.017*
Sex	0.81	0.42-1.6	0.530
Reproductive status	1.2	0.72-2	0.500
Anatomical location	1	0.45-2.2	0.990
Thoracocentesis before COP	0.75	0.41-1.4	0.350
Thoracocentesis after COP	2.5	1.4-4.7	0.002*
Blood transfusion before 4th Induction	1.5	0.51-4.1	0.480
Blood transfusion after 4th Induction	0.88	0.4-2	0.760
COP response	2.1	1.2-3.7	0.009*
Anemia before COP	2.5	1.2-5.3	0.013*
Anemia after 1st induction	1.5	0.84-2.5	0.180
Anemia after 2nd induction	1.3	0.76-2.3	0.330
Anemia after 3rd induction	0.88	0.49-1.6	0.680
Leukopenia before COP	3.4	0.46-26	0.230
Leukopenia after 1st induction	1.1	0.51-2.2	0.880
Leukopenia after 2nd induction	1.8	0.94-3.4	0.075
Leukopenia after 3rd induction	0.63	0.23-1.8	0.390
Neutropenia before COP	NA	NA	NA
Neutropenia after 1st induction	1.3	0.39-4.1	0.700
Neutropenia after 2nd induction	0.92	0.36-2.3	0.870
Neutropenia after 3rd induction	0.36	0.08-1.5	0.160
SCr before COP	0.65	0.35-1.2	0.180
SCr after 1st induction	1.9	0.96-3.9	0.065
SCr after 2nd induction	2.3	1-5.2	0.044*
SCr after 3rd induction	1.3	0.66-2.5	0.450
ALT before COP	2.3	0.84-6.6	0.110
ALT after 1st induction	2.8	1.2-6.2	0.014*
ALT after 2nd induction	1.1	0.33-3.5	0.910
ALT after 3rd induction	1	0.37-2.8	0.970

COP, cyclophosphamide, vincristine, and prednisolone; sCr, serum creatinine; ALT, alanine aminotransferase; NA, not available.

DISCUSSION

Other studies have shown that mediastinal lymphoma occurs in young-aged cats. The median age for cats in the current study was 2 years, which was similar to other studies (7, 18, 33). The bimodal age distribution in previous studies had peaks at 1 year and > 8 years (7-9, 34, 35); however, due to the high incidence of FeLV-antigen-positive cats in our research, this did not occur. Notably, domestic shorthair cats were overrepresented (more than 90%) in this study. The high prevalence of this breed might be attributable to the country (Thailand) with high FeLV prevalence and an outdoor lifestyle environment; however no specific data were collected in this study and so it was not possible to conclude breed predisposition to domestic shorthairs. Male cats had a greater ratio than female cats in this study, with the ratio of 2.1:1 being similar to other studies (5, 7, 9, 10).

However, FeLV-antigen-positive cats presented with clinical signs at the day of diagnosis that related to retroviral infection (16). The most common were respiratory disorders, loss of appetite, and anorexia, all of which impaired quality of life. All clinical signs except neurological signs decreased after the third induction of COP chemotherapy. This might have been due to the complex pathogenesis and outcomes of retroviral infection or adverse events of COP chemotherapy (22, 31).

The current study found that COP chemotherapy, the most popular protocol for feline lymphoma, was well tolerated. To our knowledge, this study had recorded the longest MST (338 days) reported in cats with mediastinal lymphoma and being FeLV-antigen-positive. In other studies, FeLV-antigen-positive cats had poor prognoses for feline lymphoma, with a median survival time of 37–134 days (10, 18, 36). On the other hand, LOPH chemotherapy was well tolerated in a recent study, with a median survival time of 214 days (19).

Of all the variables analyzed, COP response and thoracocentesis after COP significantly affected survival. Cats with a complete response (MST 379 days) had highly significantly longer survival times than those with a partial response (MST 134 days) or no response (MST 121 days), which was similar to other reports (4, 7, 8, 18, 19, 21). Cats that required thoracocentesis after COP (MST 147 days) had shorter survival times than those did not require thoracocentesis after COP (MST 373 days). If thoracocentesis had been performed after COP, the cat had partial or no response to

COP. However, COP response and thoracocentesis after COP, as prognostic factors, can only be assessed after treatment.

A prognostic factor parameter at the onset was that cats aged <4 years (MST 373 days) had longer survival times than cats aged at least 4 years old (MST 212 days). Other studies have shown that mediastinal lymphoma developed in young-aged cats (7, 18, 33) but there is no available information on FeLV-antigen-positive cats. The mechanism of FeLV-antigen-positive cats causing lymphoma is through insertion of a provirus at many different sites in the host's genome near a cellular oncogene (most commonly myc) (14, 25-27). This finding should be investigated further in terms of clinical outcomes in feline lymphoma with FeLV-antigen-positive status.

Through multivariable analysis of hematological adverse events, cats with anemia before COP, with azotemia after 2nd induction, and with elevated ALT after 1st induction had an increased hazard of death. Surprisingly, blood transfusion during treatment produced no significance change in survival time. FeLV-antigen-positive cats were found in considerable numbers (92 cats) in this study, perhaps due to FeLV infection pathophysiology, which includes bone marrow disorders (mostly anemia), neoplasia (mostly lymphoma), and immunosuppression, leading to susceptibility to secondary infections (14, 23). Elevated ALT after induction could indicate a disorder characterized by the liver's inability to metabolize substances in the body, as well as azotemia, which is defined as metabolic abnormalities caused by tumor cell cytolysis that occurs spontaneously or as a result of therapy. This result was similar to other studies (37, 38).

Because of its retrospective character, this study had several limitations. Full clinical signs and diagnostic work-up were not available for all cats. The staging of lymphoma was not identified. Only mediastinal or mediastinal plus other sites lymphoma were included. Therefore, further study may include others form of lymphoma, as well as non-infected FeLV cats.

CONCLUSION

This study showed that COP chemotherapy was well tolerated in FeLV-antigen-positive cats and resulted in compatible survival to non-infected cats. Based on weekly monitoring of clinical signs and adverse events until after the 3rd induction of COP chemotherapy, there were no life-threatening events that resulted in death. Prognostic factors were cat age at diagnosis and hematological toxicities. Cats under age 4 years had longer survival times than those aged at least 4 years. Anemia (before COP), azotemia (after the 2nd induction), and elevated ALT (after the 1st induction) had an increased hazard of death.

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