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Scientific Report

Use of a depot steroid formulation with CHOP-based protocol in the treatment of mediastinal lymphoma in cats

Cartagena Albertus, J. C.[1, 5*]; Engel Manchado, J.[1]; Romairone Duarte, A.[2]; Moise, A.[3]; Moya Garcia, S.[1]; Jones, D.[4] and Montoya-Alonso, J. A.[5]

[1]MSc, Hospital Animal Bluecare Mijas, Spain; [2]MSc, Centro de Diagnóstico Veterinario, Valladolid, Spain; [3]MSc, Sheriff Highway Newcastle, UK; [4]BSc, Northside Vets Birmingham, UK; [5]Internal Medicine, Faculty of Veterinary Medicine, University Institute for Biomedical and Health Research (IUIBS), University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

[*]***Correspondence:*** *J. C. Cartagena Albertus, MSc, Broadway Veterinary Hospital, 158 Broadway, Peterborough PE1 4DG, UK. E-mail: jcarloscartagena@yahoo.es*

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Summary

The aims of this retrospective study were to evaluate the efficacy of different steroid formulations (depot vs oral) and if the continuous administration of steroids throughout and beyond a CHOP protocol might improve the survival time and rate of cats with mediastinal lymphoma. The medical records of client-owned cats diagnosed with mediastinal lymphoma were reviewed at two veterinary referral hospitals in Spain. Cases were recruited from 2008 to 2016. Those cats with mediastinal lymphoma treated with chemotherapy were used in the collection of data including surgical procedures, chemotherapy protocol, side effects, complete remission (CR), partial remission and survival time were calculated from time of diagnosis. The median survival time (MST) of group with depot steroid was 370.7 days and all cats are still alive. The MST of group with oral steroid was 267.9 days. The survival distributions were found to be significantly different. The use of depot injectable steroids with a CHOP-based protocol seems to result in a longer survival time in patients with mediastinal lymphoma when comparing the survival distribution for those receiving oral steroids with a CHOP-based protocol. Prospective studies with a higher number of cats are warranted to investigate the utility of injectable steroids depot with a CHOP-based protocol in the treatment of mediastinal lymphoma.

Key words: Chemotherapy, Feline, Lymphoma, Mediastinum

Introduction

Lymphoma is the uncontrolled proliferation of neoplastic lymphoid cells arising in lymph nodes or other organs of the body. Some studies have been reported that the mediastinal form occurs in less than 15% of feline lymphoma cases in the USA in 1998 (Vail *et al*., 1998) and 2005 (Louwerens *et al*., 2005) and approximately 25% of cases in Australia in 1997 (Court *et al*., 1997). The mediastinal form includes involvement of the thymus and/or the mediastinal and sternal lymph nodes which may extend into the thoracic inlet (Ettinger, 2003; Vail, 2012; Shih *et al*., 2014). Cats with mediastinal lymphoma typically present for dyspnea and tachypnea secondary to pleural effusion (Ettinger, 2003; Vail, 2012; Shih *et al*., 2014). Diagnosis is commonly achieved with the identification of lymphoblastic cells on cytological evaluation of pleural fluid or fine needle aspiration (FNA) of a cranial mediastinal mass (Ettinger, 2003; Vail, 2012; Shih *et al*., 2014). Prior to the 1990s, feline leukaemia virus (FeLV) was a major risk factor for the development of feline lymphoma and leukaemia. Many of the earlier literature reports included high proportions of FeLV-infected cats, which has been shown to be associated with a poorer prognosis and also has significantly declined in prevalence since vaccination programs were introduced (Mooney *et al*., 1987; Vail *et al*., 1998; Kristal *et al*., 2001). However, even with a decrease in the overall incidence of FeLV antigenemia, the incidence of feline lymphoma is still increasing, emphasizing the relevance of this disease in veterinary oncology (Valli *et al*., 2000; Louwerens *et al*., 2005).

The immunohistochemistry for lymphoma in this study was CD3 (a marker for T cell lymphoma) and CD79a (a marker for B cell lymphoma) (Milner *et al*., 1996).

The most commonly used protocol is the CHOP-based protocol or has also been referred to as the UW-Madison protocol, UW-25, or L-ASP-VCAM. Drugs included in this protocol were vincristine, cyclophosphamide, doxorubicin, L-asparaginase and prednisone (Hahn *et al*., 1992; Garrett *et al*., 2002; Chun, 2011; Simon *et al*., 2008; Thamm, 2014).

An intramuscular steroid depot has a slow onset of action and a prolonged activity due to its moderate solubility. After the injection, the pharmacodynamic effects last several weeks or months (Ramsey, 2011).

The best way to monitor response in a patient with a mediastinal mass might require repeated thoracic radiographs, abdominal and thoracic cavity ultrasound, lymph node palpation and cytological assessment after FNA (Starrak *et al*., 1997).

The aims of this retrospective study were to evaluate the efficacy of different steroid formulations (depot vs oral) and if the continuous administration of steroids throughout and beyond a CHOP protocol might improve the survival time and rate of cats with mediastinal lymphoma.

Materials and Methods

The medical records of client-owned cats with mediastinal lymphoma were reviewed at two veterinary referral hospitals in Spain, Animal Bluecare Veterinary Hospital of Mijas and Hospital Vetersalud Dr. Moya in Torremolinos. Cases were recruited from February of 2008 until December of 2016 (Figs. 1-4, 5A-C, and 6A-B).

Inclusion criteria

Cats were included only if medical records were complete, there was a histopathological diagnosis of mediastinal lymphoma, no chemotherapy prior to referral, no other concurrent disease that could influence the survival time and no overt evidence of lymphoma at other sites.

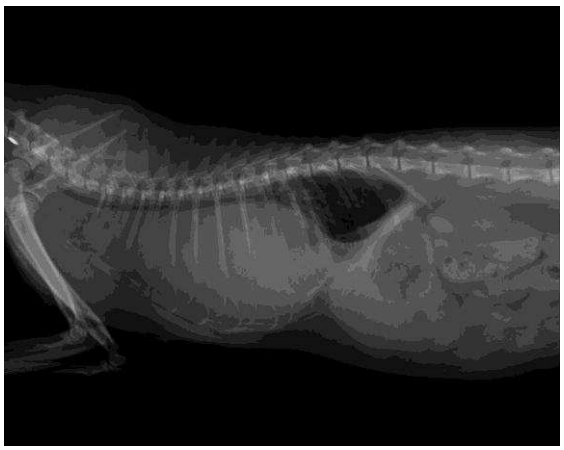

Fig. 1: Laterolateral radiographic views of thorax of a patient of this study before the beginning of the treatment. There is a mediastinal mass with a large amount of pleural effusion involving almost the complete thorax, except the caudal area

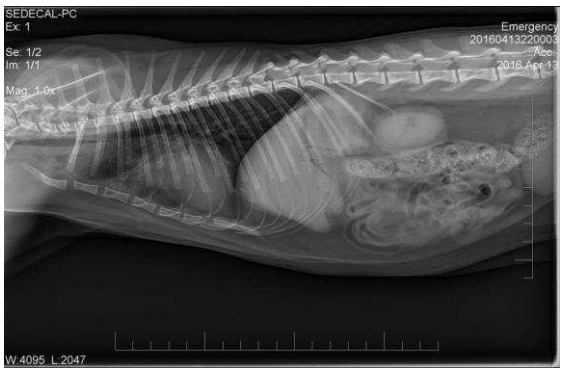

Fig. 2: Laterolateral radiographic views of thorax of a patient of this study after the beginning of the treatment. The mass and the pleural effusion visible in the previous X-ray almost disappeared after the first cycle of chemotherapy

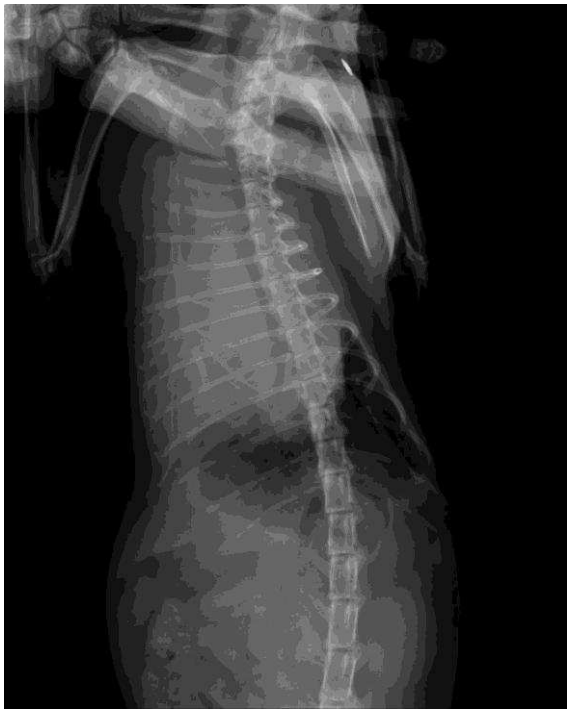

Fig. 3: Ventrodorsal radiographic views of thorax of a patient of this study before the beginning of the treatment. There is a mediastinal mass with a large amount of pleural effusion involving almost the complete thorax

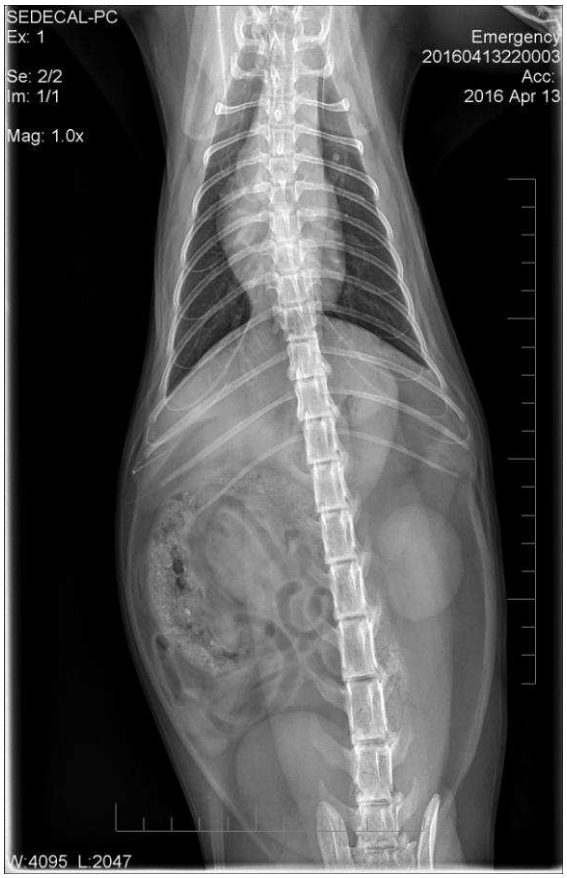

Fig. 4: Ventrodorsal radiographic views of thorax of a patient of this study after the beginning of the treatment. The pleural effusion visible in the previous X-ray disappeared after the first cycle of chemotherapy

Response criteria

Response or remission was considered complete (CR) if there was 100% reduction in size of all measurable tumors, based on assessment of tumor size following thoracic radiographs and/or thoracic ultrasound and clinical signs for at least 1 month. Partial response (PR) if there was >50% but <100% reduction in size of all measurable disease. No change in size, <50% reduction in size and increase in size of all measurable disease were classified as no response (NR). Repeat of mediastinal biopsy and histologic confirmation of remission was not performed in this clinical study.

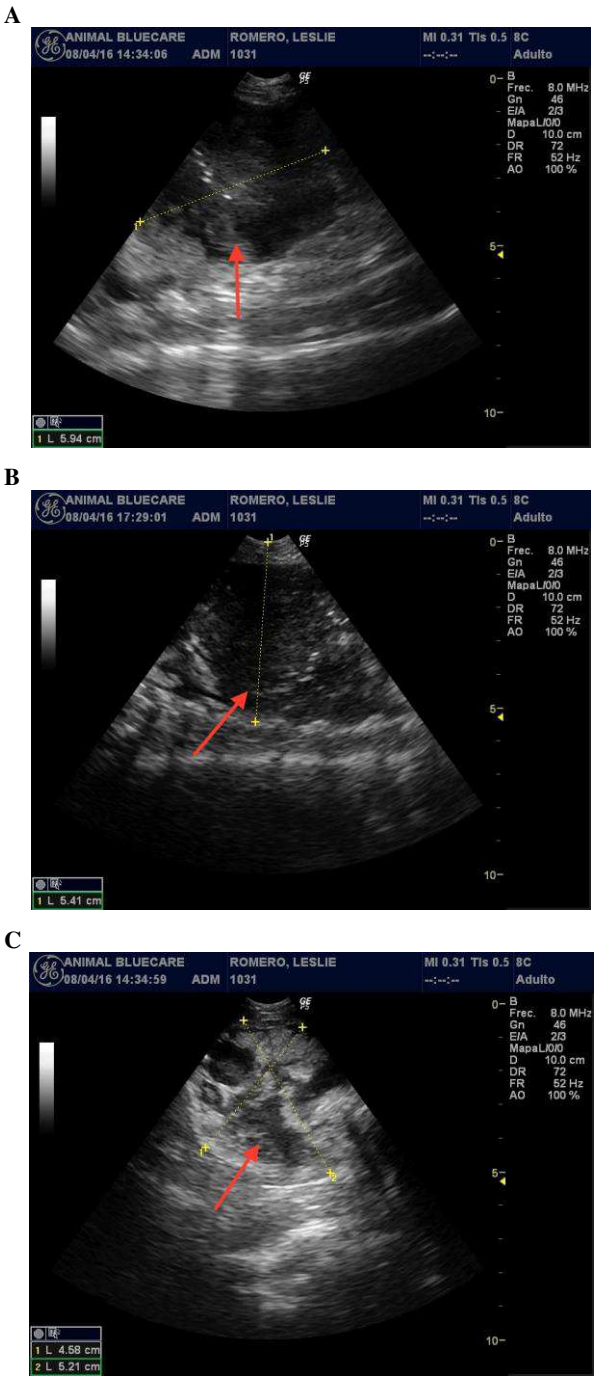

Fig. 5: Ultrasonographies of a mediastinal lymphoma of a cat of this study. The red arrow is pointing the mass

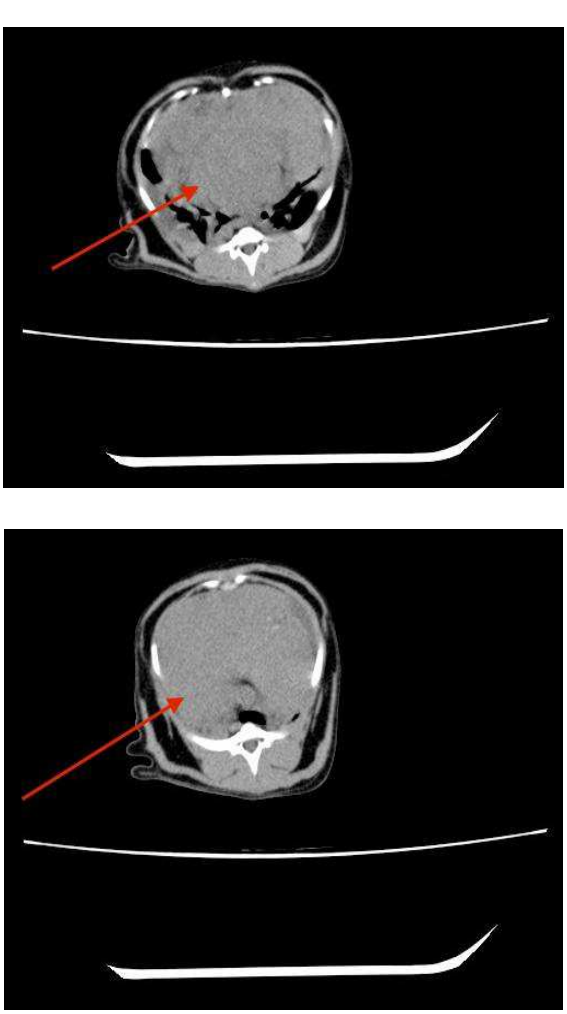

Fig. 6: CT Scan of one of the patients. Transverse planes of thorax. Red arrow pointing the mediastinal lymphoma

In all cases, diagnosis of lymphoma was confirmed with histopathological examination, whereas inmuno-chemistry revealed that the lymphocytes were positive stained for CD3 but negative for CD79a. Based on the clinical signs, cytological examination finding, histology and inmunohistochemistry, the 16 cases were diagnosed as high-grade T cell mediastinal lymphoblastic lymphomas (Figs. 7 and 8).

The sixteen cats were divided in two groups:

Group 1: Six cats were treated with a "modified" CHOP-based protocol (cyclophosphamide, doxorubicin, vincristine and a steroid depot) (Table 1). Modifications allowed in the protocol included use of injectable depot steroid instead of oral steroids at initiation of treatment and during whole survival time.

Injectable steroid depot (Methylprednisolone acetate [Depo-Medrone TM V®, Zoetis], 2 mg/kg, IM) was started in the first day of treatment repeating doses at weeks 3, 6, 9, 13, 17, 21, 25, 29, 34 and every 8 weeks during the CHOP-based protocol and during the maintenance period for each patient (Table 1).

Group 2: 10 cats treated with a "classic" CHOP-based protocol (cyclophosphamide, doxorubicin, vincristine and oral prednisone). Prednisone was used in this group

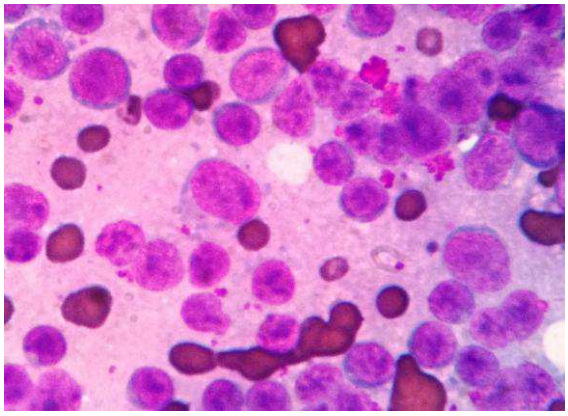

Fig. 7: Cytology of a mediastinal lymphoma (Wright-Giemsa stain, ×400) with a neoplastic proliferation of T lymphocytes characterized by a moderate amount of relatively pale cytoplasm that often contains azurophilic granules

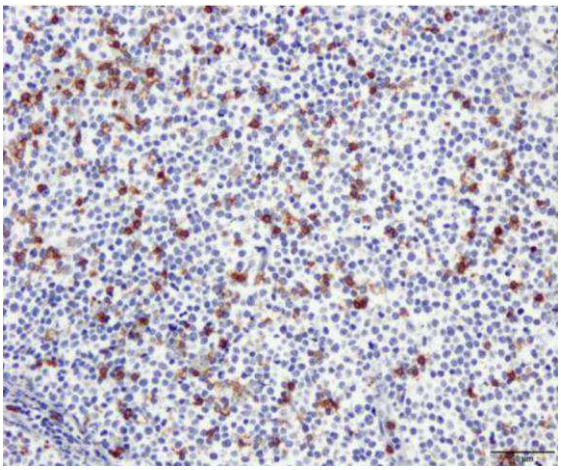

Fig. 8: Immunohistochemistry $CD3^+$ of a patient of this study. Courtesy of Histolab Veterinaria

Table 1: Treatment for group 1. Depot injectable steroids with a CHOP-based Protocol

Week 1	Vincristine 0.7 mg/m 2 IV once L-Asparaginase 400 U/kg SC steroid depot
Week 2	Cyclophosphamide 250 mg/m 2 IV once
Week 3	Vincristine 0.7 mg/m 2 IV steroid depot
Week 4	Doxorubicin 20 mg/m 2 IV once
Week 6	Vincristine 0.7 mg/m 2 IV once steroid depot
Week 7	Cyclophosphamide 250 mg/m 2 IV once
Week 8	Vincristine 0.7 mg/m 2 IV
Week 9	Doxorubicin 20 mg/m 2 IV once steroid depot
Week 11	Vincristine 0.7 mg/m 2 IV once
Week 13	Cyclophosphamide 250 mg/m 2 IV once steroid depot
Week 15	Vincristine 0.7 mg/m 2 IV once
Week 17	Doxorubicin 20 mg/m 2 IV once steroid depot
Week 19	Vincristine 0.7 mg/m 2 IV once
Week 21	Cyclophosphamide 250 mg/m 2 IV/oral once steroid depot
Week 23	Vincristine 0.7 mg/m 2 IV once
Week 25	Doxorubicin 20 mg/m 2 IV once steroid depot
Week 29	Steroid depot
Week 34	Steroid depot and every 8 weeks

Table 2: Treatment for group 2. "Classic" CHOP-based Protocol with oral steroids

Week 1	Vincristine 0.7 mg/m 2 IV once L-Asparaginase 400 U/kg SC Prednisone, 2 mg/kg PO SID
Week 2	Cyclophosphamide 250 mg/m 2 IV once Prednisone, 1.5 mg/kg PO SID
Week 3	Vincristine 0.7 mg/m 2 IV Prednisone, 1 mg/kg PO SID
Week 4	Doxorubicin 20 mg/m 2 IV once Prednisone, 0.5 mg/kg PO SID
Week 6	Vincristine 0.7 mg/m 2 IV once
Week 7	Cyclophosphamide 250 mg/m 2 IV once
Week 8	Vincristine 0.7 mg/m 2 IV
Week 9	Doxorubicin 20 mg/m 2 IV once
Week 11	Vincristine 0.7 mg/m 2 IV once
Week 13	Cyclophosphamide 250 mg/m 2 IV once
Week 15	Vincristine 0.7 mg/m 2 IV once
Week 17	Doxorubicin 20 mg/m 2 IV once
Week 19	Vincristine 0.7 mg/m2 IV once
Week 21	Cyclophosphamide 250 mg/m 2 IV/oral once
Week 23	Vincristine 0.7 mg/m 2 IV once
Week 25	Doxorubicin 20 mg/m 2 IV once

based on the following dose reduction method.

Week 1: Prednisone 2 mg/kg PO SID, week 2: Prednisone 1.5 mg/kg PO SID, week 3: Prednisone 1 mg/kg PO SID, and week 4: Prednisone 0.5 mg/kg PO SID (Table 2).

Statistical analysis

We wanted to see if survival was different between group 1 and 2, where the treatment differed by the method of administering the steroid, injectable depot versus oral.

The Kaplan-Meier product limit analysis and the log

rank (Mantel-Cox) test were used for survival analysis. Survival time was calculated from the date of diagnosis to the date of death from any cause. Cats were censored if they were alive at the end of the study. One cat in group 2 that was euthanized was considered censored, since it did not die directly as a result of the disease. The Fisher's exact test was used to compare treatment groups on the degree of remission (complete vs. partial).

All statistical analyses were performed by an independent statistical consultant.

Results

Sixteen cats characteristics are summarized in Table 3. The median age at diagnosis was 2,75 years (range1 to 6 years). The cats included 15 domestic shorthairs (93.75%) and 1 Siamese (6.25%). There were 6 spayed females (37.5%), 9 neutered males (56.25%) and one intact female (6.25%). Fifteen cats (93.75%) tested negative for FeLV antigenaemia and for FIV antibodies, one (6.25%) tested positive for FeLV antigenaemia.

Six cats received a CHOP-based protocol with injectable depot steroid (group 1) and ten cats received a CHOP-based protocol with oral steroid (group 2). The overall response rate of group 1 and 2 combined was 100% (87.5% CR, 12.5% PR and 0% NR). Of the group 1, 100% achieved a CR. The overall response rate for group 1 was 100%, 0% a PR and 0% did not respond. Of the group 2, 70% achieved a CR, 30% a PR and 0% did not respond. The overall response rate for the group 2 was 100%. A Fisher's exact test did not find statistical significance (P=0.214) when comparing the rate of CR in the group receiving injectable depot steroids (100%) with the rate for those receiving oral steroids (70%).

At the end of the study, 10/16 treated cats had died or had been euthanized and six cats were still alive. Based on data available cause of death or euthanasia was progression of lymphoma in 10 cats.

The overall median survival time (MST) was 306.4 days (range: 133-493 days). The MST of group 1 was 370.7 days and all cats are still alive. The MST of group 2 was 267.9 days. The survival distributions were found to be significantly different in group 1 and 2 using a Mantel-Cox log rank test ($\chi 2$ (1) = 10.11, P=0.001) (Tables 3-6; Fig. 9).

Table 3: Characteristics of patients included in this study

Variable	Category	Number	Percentage
Age (years)	0-3	10	62.5%
	3-6	6	37.5%
	>6	0	0%
Breed	DSH	15	93.75%
	Siamese	1	6.25%
Gender	Females	7	43.75%
	Females/Neutered	6	37.5%
	Males	9	56.25%
	Males/Neutered	9	56.25%
Vaccinated		14	87.5%
Not vaccinated		2	12.5%
FeLV/FIV	Negative	14	87.5%
FeLV	Positive	2	12.5%
FIV	Positive	0	0

Table 4: Survival analysis of groups 1 and 2

Protocol	Total N	N of events	Censored N	Percent
1	6	0	6	100.0%
2	10	9	1 *	10.0%
Overall	16	9	7	43.8%

* The one cat in group 2 that was euthanized was considered censored, since it did not die directly as a result of the disease

Table 6: Test of equality of survival distributions by protocol

	Chi-Square	df	P-value
Log rank (Mantel-Cox)	10.11	1	0.001

The survival distributions were found to be significantly different using a Mantel-Cox log rank test ($\chi 2$ (1) = 10.11, P=0.001)

Table 5: Kaplan-Meier survival estimates for groups 1 and 2

				Cumulative proportion surviving at the time			
	Protocol	Time	Status	Estimate	Std. error	No cumulative events	No remaining cases
1	1	212	0	.	.	0	5
	2	350	0	.	.	0	4
	3	368	0	.	.	0	3
	4	400	0	.	.	0	2
	5	401	0	.	.	0	1
	6	493	0			0	0
2	1	133	1	0.900	0.095	1	9
	2	199	0	.	.	1	8
	3	201	1	0.788	0.134	2	7
	4	224	1	0.675	0.155	3	6
	5	236	1	0.563	0.165	4	5
	6	276	1	0.450	0.166	5	4
	7	282	1	0.338	0.158	6	3
	8	368	1	0.225	0.140	7	2
	9	372	1	0.113	0.106	8	1
	10	388	1	0.000	0.000	9	0

Note: Estimates could not be computed for group 1 since all cases were censored (all cats were alive at the end of the observation period)

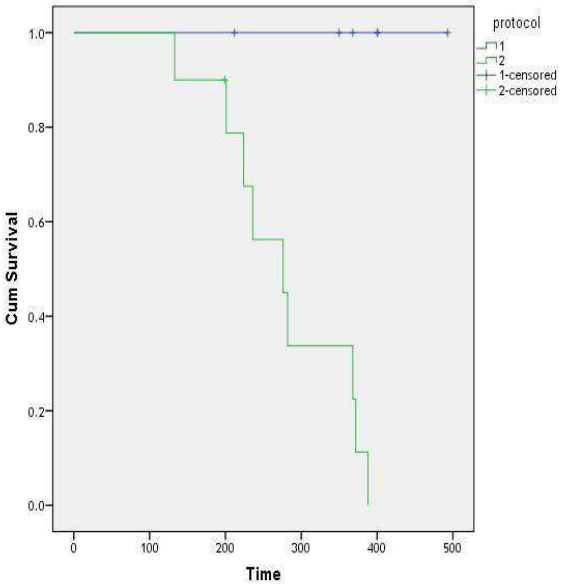

Fig. 9: Survival curve for groups 1 and 2

Discussion

Previous studies have suggested that the mediastinal form of lymphoma was typically connected with positive FeLV test result (Mooney *et al*., 1987; Mooney *et al*., 1989; Gabor *et al*., 2001; Krystal *et al*., 2001). In contrast, 1 of the 16 cats in the present study had positive FeLV test result (6.25%). Several limitations were present in this study. As well as other studies prior to this one, it was a retrospective study with few numbers of cases. There is a decrease in the proportion of the mediastinal form of feline lymphoma cases because of the introduction of FeLV vaccination in 1985 (Louwerens *et al*., 2005). The incidence of feline lymphoma is still increasing, emphasizing the relevance of this disease in veterinary oncology (Dorn *et al*., 1967; Louwerens *et al*., 2005).

Response to therapy is one of the most consistent prognostic factors noted amongst the majority of feline lymphoma studies to date (Vail, 1998; Fox, 2003; Waite, 2013). Similar to previous reports, cats that responded to therapy achieving a CR survived significantly longer MST (Mooney *et al*., 1987). It is possible to predict the long-term outcome for many patients in the first 4-6 weeks of a CHOP-based chemotherapy protocol. The patient will receive five of the most commonly used drugs with antitumor activity against lymphoma after 4-6 weeks of therapy. At the end of 6 weeks, it is possible to make an assessment of protocol efficacy and potential drug toxicity/tolerance. The cats with an initial CR to therapy lived longer (12-18 months) versus the cats with a PR to therapy (6-8 months). The CR rate achieved with the CHOP-based protocol and the injectable depot steroid (group 1) was 100% and all cats are alive at the moment of writing this report and no important side effects were reported. The CR rate achieved with the "classic" CHOP-based protocol was 70%.

The response rate to the CHOP-based protocol reported in previous studies was 66.7% for mediastinal lymphoma (Fabrizio *et al*., 2013).

A Fisher's exact test did not find statistical significance when comparing the rate of CR in the group 1 (100%) with the rate in the group 2 (70%), because of the reduced number of cases.

The MST of group 1 was 370.7 days and all cats are still alive. The MST of group 2 was 267.9 days. The survival distributions were found to be significantly different using a Mantel-Cox log rank test. The use of depot injectable steroids with a based CHOP protocol seems to result in a longer survival time in patients with mediastinal lymphoma when comparing the survival distribution for those receiving oral steroids with a based CHOP protocol.

In conclusion, the use of depot injectable steroids combined with a CHOP-based protocol throughout and beyond a CHOP protocol seems to result in a longer survival time in patients with mediastinal lymphoma. However, these preliminary results need to be interpreted with caution because of the retrospective nature of this study and the small amount of data available. Prospective studies with a higher number of cases are warranted to investigate the utility of injectable steroids depot with CHOP-based protocol in the treatment of mediastinal lymphoma.

Conflict of interest

This study and its authors do not have any potential conflicts of interest to declare. Funding this research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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