Veterinary Oncology

RESEARCH

Open Access

Outcomes and prognostic factors in canine T cell lymphoma treated with lomustine, vincristine, cyclophosphamide, and prednisone chemotherapy

Anat Einhorn[1*], Gillian Dank[1], Erez Hanael[1], Michael S. Kent[2], Craig A. Clifford[3], Asia Dunaevich[1] and Einat Yas[1]

Abstract

Background The most common first-line treatment for canine lymphoma is a chemotherapy protocol that includes cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). Canine high-grade T-cell lymphoma has been found to have a significantly poorer prognosis than high grade B-cell lymphoma. Several studies have investigated alternative protocols for non-indolent T-cell lymphoma. This retrospective study investigated using a cyclophosphamide, lomustine, vincristine, and prednisone protocol (CLOP) for naïve non-indolent T-cell lymphoma patients.

Methods In this retrospective study, medical records of dogs treated for non-indolent T-cell lymphoma at a veterinary teaching hospital from 2017 to 2022 were reviewed. Response rate, toxicity, progression-free survival and survival time were calculated. Factors potentially related to prognosis were statistically analyzed.

Results Twenty-six dogs were included in the study. The median progression-free survival (PFS) time was 166 days (95% CI 119–213). The median overall survival time (OST) for the whole study group was 318 days (95% CI 239–374). Twenty-four dogs experienced gastrointestinal adverse events during the protocol, with 79% of them being grade 1 or 2 as per VCOG-CTCAE v2.

Conclusions This protocol has shown similar median PFS time and OST compared with previous studies for canine non-indolent T-cell lymphoma treated with CHOP, along with minimal toxicity, and suggests the inclusion of lomustine in first-line chemotherapy protocol against canine non-indolent T-cell lymphoma may be beneficial.

Keywords Canine, Chemotherapy, Lomustine, Lymphoma, Treatment

Background

Lymphoma is the most common hematopoietic malignancy in dogs [1]. Classification of canine lymphoma is based on anatomic location, histologic criteria and immunophenotypic characteristics [1]. More than 24 categories of lymphoma have been described, but most commonly are divided into B-cell or T-cell phenotypes [1].

The most common first-line treatment for canine lymphoma is a chemotherapy protocol that includes cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) [1]. Several studies have reported

*Correspondence:
Anat Einhorn
anat.einhorn@gmail.com
[1] Department of Veterinary Oncology, Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel
[2] Department of Surgical and Radiological Sciences, UC Davis School of Veterinary Medicine, Davis, CA, USA
[3] BluePearl Malvern, Malvern, PA 19355, USA

median progression-free survival (PFS) times of 140–282 days and median overall survival times (OST) of 257–397 days for dogs treated with CHOP for multicentric lymphoma, but differences between the immunophenotypes were not investigated [2–5].

Canine non-indolent T-cell lymphoma was found to have a significantly poorer prognosis than B-cell lymphoma when treated with the same protocol [4, 6, 7].

In two previous studies investigating the use of CHOP as a first-line treatment for non-indolent T-cell lymphoma, the median first remission durations were 104 and 133 days, and OST was 235 days with one study not reporting OST [8, 9].

There have been several studies investigating alternative protocols for T-cell lymphoma such as L-asparaginase and CHOP (L-CHOP) and AT-005 monoclonal antibody; mechlorethamine, vincristine, procarbazine and prednisone (MOPP); lomustine, vincristine, procarbazine and prednisolone (LOPP); cyclophosphamide, vincristine, epirubicin and prednisolone (CEOP); cyclophosphamide, vincristine, epirubicin and lomustine (LEOP); vincristine, L-asparaginase, cyclophosphamide, lomustine, doxorubicin, prednisolone, procarbazine and mechlorethamine (VELCAP-TSC) and various single agent protocols. In these studies, the PFS and OST for dogs with T-cell lymphoma ranged from 97–431 and 202–507 days, respectively [8–17]. Of the studies mentioned above the only prospective study was the L-CHOP and AT-005 monoclonal antibody study. In this study all dogs received a 19-week L-CHOP chemotherapy protocol with randomization into placebo or AT-005 groups. The median PFS was disappointing at 103 days in the placebo group versus 64 days in the AT-005 group [17].

Some studies showed that doxorubicin and vincristine drugs are less effective on T-cell lymphomas, in vitro, due to enhanced efflux from the tumor cells by high levels of p-glycoprotein, which is a product of the MDR1 gene [18–22]. In these cases, replacing doxorubicin with an alkylating agent, which is not affected by MDR1, such as lomustine, could improve the PFS and OST of patients.

Procarbazine (used in the LOPP, MOPP, and VELCAP-TSC protocols) has increased cost, is not always as available as lomustine, and requires daily administration by the owners. For these two reasons, we chose to investigate a protocol similar to CHOP where doxorubicin is replaced by lomustine.

This study aims to retrospectively investigate the use of a cyclophosphamide, lomustine, vincristine, and prednisone protocol (CLOP) treatment in dogs with naïve T-cell lymphoma patients and identify prognostic factors.

Methods

Study population

The medical records of dogs diagnosed with lymphoma at the Koret School of Veterinary Medicine, Hebrew University of Jerusalem, were retrospectively reviewed (years 2017-2022). Patients with multicentric non-indolent lymphoma were included in the study if they had cytological or histologic diagnosis of lymphoma and a T-cell immunophenotype. Dogs were excluded in the following cases: a definitive diagnosis was not achieved, immunophenotyping was not available, cytological or histopathological morphological characteristics were suggestive of a "low grade" or T-zone lymphoma (TZL), dogs with stage I or stage II lymphoma, dogs with gastrointestinal or skin involvement, primary hepato-splenic lymphoma, and dogs with a history of prior chemotherapy treatment. Clinical records were reviewed and follow-up data was obtained from existing medical records and from referring veterinarians by phone calls.

Diagnosis and staging

Cytopathological evaluation of lymph nodes was used to diagnose lymphoma. T-cell lymphomas were diagnosed by immunoreactivity with CD3 antibody and lack of reactivity with PAX5 [23]. The same immunoreactivity was used to diagnose histopathological specimen. All immunoreactivity tests were performed by the same diagnostic laboratory (Eastern VetPath laboratories, 211 Perry Pkwy, Suite 4, Gaithersburg, MD 20877, Unites States).

Clinical stage and substage were classified based on the World Health Organization (WHO) criteria for canine lymphoma [24]. Dogs presenting with clinical signs associated with systemic illness were classified as substage b at the discretion of the attending clinician (dogs with only mild lethargy were classified as substage a. All other clinical signs were classified as substage b). Dogs that did not undergone abdominal ultrasound were categorized as stage III lymphomas. Stage IV lymphomas were differentiated from stage III by atypical sonographic anomalies of spleen and liver. Presence of neoplastic lymphocytes in peripheral blood smears was used to classify cases as stage V lymphoma according to WHO classification [24]. Bone marrow aspirates were not performed. Studies have shown that bone marrow involvement can occur in the absence of circulating neoplastic lymphocytosis [25]. However, in this study in cases without evidence of lymphocytosis, it was assumed that there was no bone marrow involvement.

All patients had a physical examination, complete blood count (CBC), blood smear review, and biochemical analysis performed prior to initiation of treatment and results were analyzed. Blood samples for CBC (Advia 120 or 2120, Siemens, Erlangen, Germany) and serum

Table 1 CLOP chemotherapy treatment protocol

	Week															
Protocol	1	2	3	4	6	7	8	9	11	13	15	17	19	21	23	25
Vincristine (0.7 mg/m2), IV	•		•		•		•		•		•		•		•	
Cyclophosphamide (200 mg/m2), PO + furosemide (1 mg/kg twice)		•				•				•				•		
Lomustine (70 mg/m2), PO				•				•				•				•
Prednisone (tapering dose)	40 mg/m^2	30 mg/m^2	20 mg/m^2	10 mg/m^2												

chemistry (Cobas 6000, Roche, Mannheim, Germany) were collected in potassium-EDTA and plain tubes with gel separators, respectively, and analyzed within 60 min from collection.

Dogs were considered anemic if the hematocrit (HCT) was less than 37%. Neutropenia/neutrophilia were considered if neutrophil count was below/above the reference range ($3.9–8.0 \times 10^9$/L). Monocytopenia/monocytosis were considered if monocyte count was below/above the reference range ($0.2–1.1 \times 10^9$/L). Dogs were considered thrombocytopenic if the platelet (PLT) count was less than 140×10^9/L. Lymphocytosis was considered if lymphocyte count was above the reference range (4.1×10^9/L).

Dogs were considered hypercalcemic if total calcium concentration was above the reference range (11.5 mg/dL), and ionized calcium concentration was above the reference range (1.35 mmol/L). If ionized calcium concentration was not available, hypercalcemia was defined by an increased total calcium concentration. Hypoalbuminemia was diagnosed when the albumin concentration was below 30 g/L.

Hyperglobulinemia was diagnosed when the globulin concentration was above 46 g/L.

ALT was considered elevated if its concentration was above 67 IU/L. Diagnostic imaging modalities and findings were recorded for each case. If internal lymph nodes were enlarged, they were classified as being involved.

Treatment

The CLOP protocol was administered to the dogs included in this study over a-6 month period. The protocol used is detailed in Table 1. Silybin/S-adenosy-L-methionine was not administered to any patient.

All patients had a complete physical examination, a CBC, a blood smear, and the owners were inquired about any adverse events (AEs) prior to each treatment dose to assess evidence of toxicity. Dose reductions were made due to AEs at the discretion of the attending clinician.

Treatment was delayed if neutrophil count was $< 3*10^9$/L or platelet count was $< 75000*10^9$/L, and if reported AEs were still present at presentation.

L-asparaginase was administered at a dose of 400IU/Kg, up to a maximum amount of 10,000 IU/dog for dogs of substage b having severe enough clinical signs to raise concern of deterioration using vincristine at the discretion of the attending clinician.

After completion of the 25-week CLOP protocol, monthly recheck appointments were advised to monitor remission status. This included palpation of lymph nodes in all patients. Additional testing depended on the primary presentation of the patient and could include CBC, blood smear, biochemistry panel, thoracic radiographs, and/or abdominal ultrasound, at the discretion of the attending clinician.

Response

Response to first-line and rescue chemotherapy treatments was based on the Veterinary Cooperative Oncology Group (VCOG) response evaluation criteria for peripheral nodal lymphoma [26]. Cases were classified as being in complete remission (CR) when lymph nodes (both peripheral and/or internal) had returned to normal size; partial remission (PR) when lymph nodes remained enlarged but had reduced in size by at least 30% and no new lesions were recognized; progressive disease (PD) was used for occurrence of new lesions or an increase in size of enlarged lymph nodes by at least 20%; and stable disease (SD) as a change in size of lymph nodes which was not sufficient to be classified as PD or PR with no occurrence of new lesions. Remission status was assessed after every cycle of the protocol.

Demographic data, treatment protocols, clinical response, toxicity from the chemotherapy protocol, date of progression, rescue treatments, date and cause of death were recorded for each patient.

Toxicity

VCOG common terminology criteria for adverse events (CTCAE) v2 were used to grade the severity of AEs and toxicity. Clinical records and blood results were examined retrospectively for the evidence of AEs including myelosuppression (neutrophils, thrombocytopenia), gastrointestinal toxicity (anorexia/hyporexia, nausea, vomiting, and diarrhea), and elevated alanine transaminase (ALT) at each follow up appointment. AEs were graded from 1 to 5 as per VCOG-CTCAE v2 [27].

Statistical analysis

Statistical analysis was done using a commercially available software program (STATA 14.2, Stata Corporation, College Station, TX USA). Categorical data was reported as number and percentage. All continuous data was assessed for normality using the Shapiro–wilk test. If data was normally distributed, then means and standard deviation was reported. If data was not normally distributed, then medians and range was reported. The Kaplan–Meier method was used to estimate median progression free survival (PFS) time and overall survival time (OST). For survival analysis time was defined as between the first treatment day and day of progression or death. For censoring, all deaths were considered events, with dogs lost to follow-up or alive at analysis censored. Median time to event along with 95% confidence intervals were reported. To identify differences in estimated survival times between categorical variables, a log rank test was used. To identify differences in survival times for continuous variables, a Cox regression was done. A p value < 0.05 was considered statistically significant.

Results

Patient characteristics

Twenty-six dogs met inclusion criteria and were included in the study. Information regarding signalment, weight, combined stage, immunophenotyping, calcium status, pre-treatment with steroids, and L-asparaginase administration is presented in Table 2. The most common breeds in this study were mixed breed dogs (27%) and Dogue de Bordeaux (23%).

Twenty-five were diagnosed using cytology and immunocytochemistry, one dog was diagnosed using histopathology and immunohistochemistry. All dogs were CD3 positive and PAX5 negative. Full staging was not performed for all dogs. Nineteen (73%) dogs had an abdominal ultrasound, five (19%) dogs had thoracic radiographs, one (4%) dog had positive liver cytology with an abnormal liver appearance on ultrasound, and two (8%) dogs had positive splenic cytology with an abnormal splenic appearance on ultrasound.

Table 2 Population characteristics

Parameter		
Age (years)	Mean (±)	7.15 (1.96)
Sex	Male neutered (n)	8
	Male intact (n)	7
	Female neutered (n)	9
	Female intact (n)	2
Weight (Kg)	Mean (±)	33.08 (15.35)
Breed	Mixed breed	7 (28%)
	Dogue de Bordeaux	6 (23%)
	Cane Corso	2 (7%)
	Golden retriever	2 (7%)
	Boxer	2 (7%)
	Other (one each)	7 (28%)
Combined stage [a]	IIIa	7 (27%)
	IIIb	10 (38%)
	IVa	1 (4%)
	IVb	6 (23%)
	V	2 (8%)
Immunophenotyping	Immunohistochemistry	1 (4%)
	Immunocytochemistry	25 (96%)
Calcium status	Elevated	14 (54%)
	Normal	12 (46%)
Pre-treatment with steroids	Yes	8 (31%)
	No	18 (69%)
L-asparaginase administered at first treatment	Yes	14 (54%)
	No	12 (46%)

[a] Combined stage- WHO clinical stage + substage

All dogs had blood smear evaluation, only two (8%) dogs were positive for circulating lymphoblasts, both with less than 10×10^9/L lymphocytes on complete blood count, were categorized as stage V.

Eight dogs (31%) had mediastinal involvement, diagnosed using thoracic radiography and/or ultrasound, but cytology was not performed.

One dog (4%) had pulmonary involvement, diagnosed using thoracic radiography and suitable clinical signs. Since no cytology and/or bronchoalveolar lavage were obtained, this dog was classified as stage IV.

According to WHO staging system 17 dogs (65%) were categorized as stage III, 7 (27%) as stage IV and 2 (8%) as stage V. Eight dogs (31%) were of substage a and the remaining 18 dogs (69%) were considered substage b.

Seven dogs (27%) were anemic at the initial diagnosis. Mean hematocrit was 43.37% (± 9.06). Twelve dogs (46%) were thrombocytopenic at initial diagnosis. Median platelet count was 152×10^9/L (range $27–432 \times 10^9$/L). Three dogs (11.5%) had lymphocytosis at presentations.

Median lymphocyte count was 1.6×10^9/L (range 0.47–8.26×10^9/L). Eight dogs (28%) were neutropenic, and eight dogs (28%) had neutrophilia. Median neutrophil count was 5.49×10^9/L (range 2.31–120.6×10^9/L). Three dogs (10%) had monocytopenia, and five dogs (17%) had monocytosis. Median monocyte count was 0.52×10^9/L (range 0.12–4.73×10^9/L). Fourteen dogs (54%) were hypercalcemic before initiation of treatment. Median total calcium was 12 mg/dL (range 6.8–16.9 mg/dL), median ionized calcium was 1.79 mmol/L (range 0.94–2.22 mmol/L). Seven dogs (27%) had hypoalbuminemia at first presentation. The mean albumin concentration was 33.27 g/L (± 5.91). Three dogs (11.5%) had hyperglobulinemia at presentation. The mean globulin concentration was 30.11 g/L (± 9.83). Nine dogs (34.5%) had elevated ALT at the initial diagnosis. The median ALT concentration was 53 IU/L (range 19–565 IU/L). Median creatinine was 1.27 mg/dL (range 0.41–3.29 mg/dL).

Treatment response/toxicity, survival and prognostic factors

Fourteen dogs (54%) received L-asparaginase at first presentation. Eight dogs (31%) were pretreated with prednisone for 6.75 days (± 2.66, range 3–11 days) prior to protocol initiation by the referring veterinarian while waiting for an oncology appointment. The median total cumulative vincristine, cyclophosphamide, and lomustine doses were 4.55 mg/m^2 (range 0.7–5.6), 800 mg/m^2 (range 0–800), and 280 mg/m^2 (range 70–360), respectively. Three dogs had an initial vincristine dose reduction (from 0.7 mg/m2 to 0.6 mg/m2) due to substage b, not necessitating L-asparaginase administration but enough to raise the concern for vincristine AEs and were done at the attending clinician's discretion.

Twenty-four dogs experienced gastrointestinal adverse events during the protocol, with 79% of them being grade 1 or 2.

Six dogs were switched from vincristine to vinblastine due to gastrointestinal AEs after the first cycle, and 1 dog received chlorambucil instead of cyclophosphamide, due to gastrointestinal AEs after the first cycle, as well [1, 28]. No dog had sterile hemorrhagic cystitis, nor chemotherapy induced thrombocytopenia during the protocol.

Thirteen dogs (50%) completed the protocol. Twelve dogs did not complete the protocol due to disease progression and one dog due to toxicity. The best response for twenty-two dogs was a CR, thirteen of them received L-asparaginase treatment before initiating the protocol. The best response for the remaining four was a PR, two of them received L-asparaginase treatment before initiating the protocol.

AEs are illustrated in Table 3.

Table 3 Adverse events

		VCOG-CTCAE v2 Grade			
Total adverse events (*n* = 98)		**1**	**2**	**3**	**4**
Vincristine	Gastrointestinal	7	10	4	0
	Neutropenia	22	3	3	3
Cyclophosphamide	Gastrointestinal	8	1	0	0
	Neutropenia	9	1	0	1
	Hemorrhagic cystitis	0	0	0	0
Lomustine	Gastrointestinal	2	2	2	0
	Neutropenia	8	2	4	3

Sixteen dogs (61.5%) had no dose reductions, 3 dogs (11.5%) had one dose reduction, 4 dogs (15.5%) had two dose reductions, and 3 dogs (11.5%) had six dose reductions.

Three dogs (11%) had no dose delays, 7 dogs (27%) had one dose delay, 6 dogs (23%) had two dose delays, 6 dogs (23%) had three dose delays, 2 dogs (8%) had five dose delays, 1 dog (4%) had six dose delays, and 1 dog (4%) had eight dose delays. Dose delays were between 2 to 7 days.

Five dogs (19%) were hospitalized during their first protocol. Four of those dogs were hospitalized due to gastrointestinal AEs. One dog was hospitalized after lomustine administration, and three dogs after vincristine administration. One dog was hospitalized due to severe neutropenia seven days after the first lomustine administration. All dogs were discharged after 24–48 h.

Eleven dogs had elevated ALT on the last follow-up. Only two of them had an elevated ALT of grade 3, the rest were of grade 1 or 2.

Nineteen patients had rescue chemotherapy due to progression. The median number of rescue protocols was two (range 1–4). Five patients had one rescue protocol, six patients had two rescue protocols, six patients had three rescue protocols, and two patients had four rescue protocols. Rescue protocols were not standardized and consisted of single-agent doxorubicin ($n = 10$), CHOP-based protocols ($n = 2$), single-agent lomustine ($n = 8$), single-agent L-asparaginase ($n = 14$), LOPP ($n = 4$), and single-agent mitoxantrone ($n = 1$).

Progression free survival and prognostic factors

After induction treatment, the median PFS was 166 days (95% CI 119–213) and presented in Fig. 1. One dog had lost remission at 678 days and (was alive or lost to follow up at 732 days).

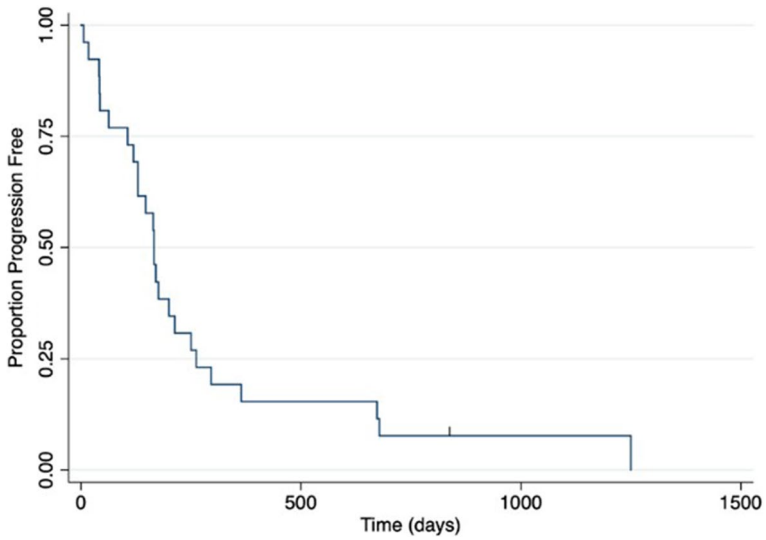

Fig. 1 Progression free survival. Legend: Kaplan–Meier curve for PFS for all dogs. PFS was 166 days (95% CI 119–213). Dogs still alive or lost to follow up are indicated by tick marks

Univariable analysis

Age ($p=0.83$), sex ($p=0.16$), breed ($p=0.30$), weight ($p=0.24$), substage ($p=0.99$), clinicopathological parameters upon presentation [including anemia ($p=0.37$), HCT ($p=0.65$), PLT count ($p=0.20$), thrombocytopenia ($p=0.68$), neutrophil count ($p=0.11$), monocytosis ($P=0.11$), lymphocyte count ($p=0.13$), total and ionized calcium concentration ($p=0.79$ and $p=0.64$, respectively), hypercalcemia ($p=0.99$), albumin and globulin concentration ($p=0.59$ and $p=0.30$, respectively), hypoalbuminemia ($p=0.75$), creatinine concentration ($p=0.69$), elevated ALT ($p=0.18$) and ALT concentration ($p=0.97$)], extranodal involvement (mediastinal or pulmonary) ($p=0.34$), pre-treatment with steroids ($p=0.38$), number of days on steroids prior to treatment ($p=0.08$), L-asparaginase induction ($p=0.15$), switching from vincristine to vinblastine ($p=0.96$), number of dose reductions ($p=0.37$) and delays ($p=0.33$), and number of hospitalizations during protocol ($p=0.44$) were not prognostic for PFS.

Factors significantly associated with shorter PFS were lymphocytosis upon presentation (median PFS 42 days, $p<0.001$), monocyte count upon presentation [(HR 2.47 (95% CI 1.32 – 4.64) ($p=0.008$)], hyperglobulinemia upon presentation (median PFS 63 days, $P=0.003$), and circulating lymphoblasts on peripheral blood smear ($p=0.05$).

Overall survival

Median overall survival time was 318 days (95% CI 239–374) and presented in Fig. 2. Two dogs were still alive at 732 days and 1,463 days.

Univariable analysis

Age ($p=0.12$), sex ($p=0.18$), breed ($p=0.42$), weight ($p=0.29$), substage ($p=0.81$), clinicopathological parameters upon presentation [including anemia ($p=0.89$), HCT ($p=0.61$), PLT count ($p=0.07$), thrombocytopenia ($p=0.17$), neutrophil count ($p=0.62$), lymphocyte count ($p=0.08$), lymphocytosis ($p=0.45$), total and ionized calcium concentration ($p=0.79$ and $p=0.57$, respectively), hypercalcemia ($p=0.37$), albumin and globulin concentration ($p=0.59$ and $p=0.33$, respectively), hypoalbuminemia ($p=0.26$), creatinine concentration ($p=0.69$), elevated ALT ($p=0.78$) and ALT concentration ($p=0.39$)], extranodal involvement (pulmonary and/or mediastinal) ($p=0.82$), pre-treatment with steroids ($p=0.54$), number of days on steroids prior to treatment ($p=0.05$), L-asparaginase induction ($p=0.15$), switching from vincristine to vinblastine ($p=0.79$), number of dose reductions ($p=0.69$) and delays ($p=0.97$), and number of hospitalizations during protocol ($p=0.95$), and number of rescue protocols ($p=0.44$) were not prognostic for OST.

Factors significantly associated with shorter OST included circulating lymphoblasts on peripheral blood smear ($p=0.01$), monocyte count upon presentation [HR 1.78 (95% CI 1.09 – 2.94) ($p=0.04$)], monocytosis

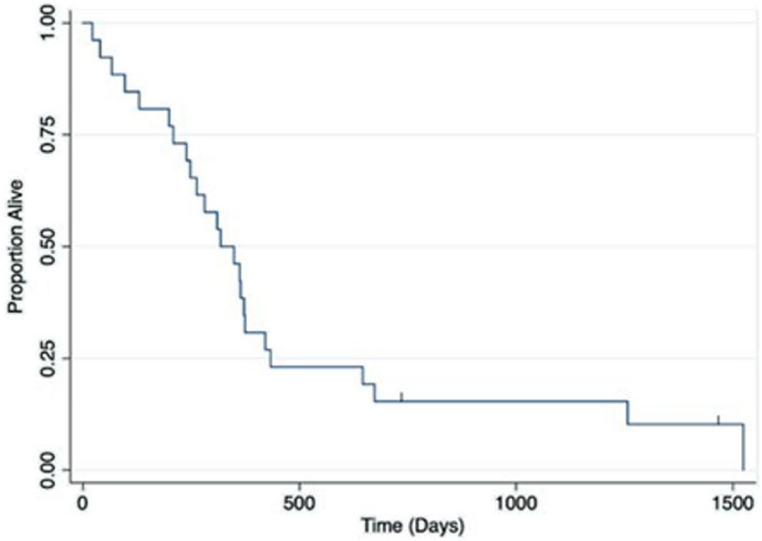

Fig. 2 Overall survival. Legend: Kaplan–Meier curve for OST for all dogs 318 days (95% CI 239–374). Dogs alive at the time of analysis or lost to follow-up were censored and indicated by tick marks

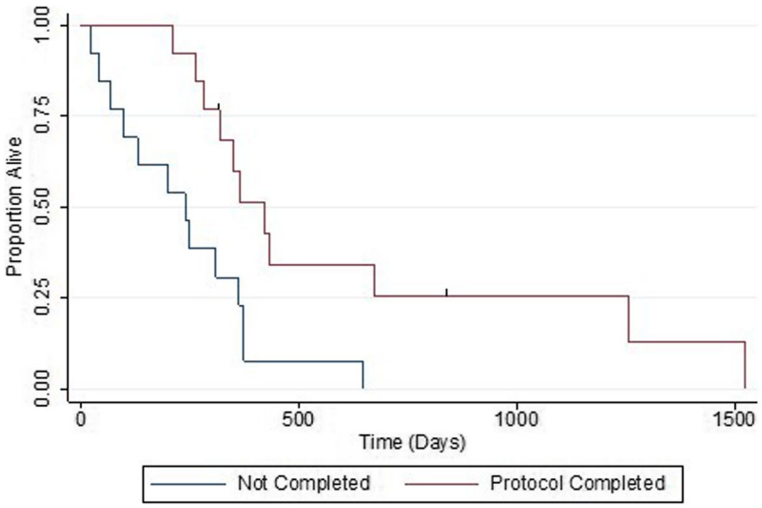

Fig. 3 OST for dogs that completed the protocol vs. those dogs which did not. Legend: Kaplan–Meier curve for OST for dogs that completed the protocol vs. those dogs which did not. ($P=0.005$). Dogs that were alive at the time of analysis or that were lost to follow up were censored and indicated by a tick mark

upon presentation ($p=0.03$), and hyperglobulinemia upon presentation ($p=0.04$).

Completing the protocol was found as a positive prognostic factor for increased OST ($p=0.005$) (Fig. 3).

Discussion

The purpose of this study was to evaluate the use of CLOP chemotherapy protocol in dogs with non-indolent T-cell lymphoma. This specific protocol had yet to be reported. The secondary goals were to identify prognostic factors for these dogs, associated with PFS and OST and to evaluate for toxicity. For the dogs included in this study, we found a high response rate (100%) similar to conventional CHOP or lomustine based protocols [3, 4, 7, 8, 11, 14, 15].

We also found that the response was of similar duration (PFS 166 days, OST 318 days) compared to historically reported T-cell lymphoma treated with conventional CHOP protocol [3, 4, 6–8].

The median PFS of 166 days (95% CI 119–213 days) and the median OST of 318 days (95% CI 239–374 days)

in this study were similar to those reported in three previous studies of canine T cell lymphoma: median disease free interval (DFI) of 176 days (range 0–1745 days) and median OST of 323 days (range 51–1758 days) for patients receiving LOPP, median PFS of 175 days (95% CI 119–231 days) for patients receiving VELCAP-TS, and OST of 327 days for patients receiving LEOP [11, 14, 15].

Our results are slightly better than those found by Blaxhill et al. using LOPP protocol with a median PFS of 118 days (range 7–2302 days) and median OST of 202 days (range 8–2302 days) [12].

On the contrary, our results are inferior to the results of Morgan et al. also using LOPP protocol with a median PFS time of 431 days (range 1–2,714 days), and median OST of 507 days (range 6–2,714 days) [10].

There are several additional advantages to the use of CLOP over LOPP. Procarbazine (used in the LOPP protocol) has increased cost, is not always as available as lomustine, requires daily administration by the owners and the combination with lomustine in previous LOPP protocols have led to reported high percentage of dogs suffering from myelosuppression [10–12].

Compared to a previous study using the LOPP protocol, this protocol had fewer AEs, and the majority of those events with the current protocol were of VCOG-CTCAE v2 grade 1 and 2 [12]. Although fewer AEs were reported, a higher number of hospitalizations (19%) were documented compared to LOPP protocols, which reported 7%-12.9% of dogs hospitalized during the protocol [10–12]. The number of hospitalizations was similar in our study to number of hospitalizations with CHOP (11.5%-27%) and MOPP (17.3%-20%) protocols [4, 6, 7, 9, 13]. In those protocols neutropenia was the main reason for hospitalization, while in our study, the main reason was gastrointestinal AEs. It is essential to notice that most gastrointestinal AEs occurred after vincristine which is included in all those protocols, and not after lomustine administration.

There was a high percentage of dose delays in our study, which might be explained due to a rather conservative cut-off, where treatment was delayed if the neutrophil count was less than 3×10^9/L. Recent evidence suggests a lower neutrophil count is adequate to give chemotherapy, and this cut-off should be used in further studies [29].

Dogue de Bordeaux ($n=6$, 23%) was the most common breed reported in this study. This is unsurprising as they are a breed known to be predisposed to T-cell lymphoma [30–32].

In our study, mediastinal or pulmonary involvement were not found to be significantly associated with shorter PFS ($P=0.34$). Most cases were mediastinal, and only one case with pulmonary involvement based on radiographic appearance. Mediastinal lymphadenopathy was historically reported to be associated with shorter PFS [33, 34].

Hypercalcaemia has previously been reported to be a negative prognostic indicator for treating canine lymphoma [2, 3, 13, 35, 36]. In our study, hypercalcemia was not found to be prognostic for PFS nor OST per previous studies. Still, the small population size could have influenced the results [4, 11, 30].

Elevated ALT at first diagnosis was considered as secondary to lymphoma and thus dogs with elevated ALT were not excluded from the study. Since most dogs with elevated ALT had grade 1 or 2 elevations, no hepatic protectants were administered.

Since splenic/hepatic cytology were not obtained for most dogs, the stage categorization could have been redefined for some of the cases from stage IV to stage III [37]. This stage migration does not have a significant impact on the patients' treatment and outcome [37].

Among factors significantly associated with shorter OST we found circulating lymphoblasts on peripheral blood smear to be prognostic ($p=0.01$).

It should be noted, though, that only two dogs in our study had circulating lymphoblasts, thus, interpretation of the true prognostic impact should be made with caution. On both of them the lymphocyte count on CBC was less than 10×10^9/L. Also, flow cytometry and/or bone marrow biopsy were not obtained on them. Although leukemia could not have been ruled out, we considered these findings as lymphoma stage V. A limitation of the study is that without bone marrow examinations it is likely that we could be missing dogs with early bone marrow involvement. This is even more likely since 12 cases presented with evidence of thrombocytopenia and 7 cases presented with neutropenia which could indicate early bone marrow involvement without circulating lymphoblasts. None of the cases presenting with thrombocytopenia/neutropenia also had circulating lymphoblasts on the CBC evaluation.

Additional limitations of this study include those attributed to the retrospective study design, and the small size of the study's population, incomplete staging for some of the dogs, diagnosis based on cytology and immunoreactivity and not on histopathology and/or flow cytometry.

Larger controlled prospective double-arm studies are necessary to assess the outcome and prognostic indicators for non-indolent multicentric T-cell lymphoma in dogs treated with the CLOP protocol and to support the findings of this study. The CLOP chemotherapy protocol could be considered for first-line treatment of naïve non-indolent T-cell lymphoma.

Conclusions

Our protocol has shown similar median PFS time and OST compared with previous studies for canine non-indolent T-cell lymphoma treated with CHOP, along with minimal toxicity, and suggests the inclusion of lomustine in first-line chemotherapy protocol against canine non-indolent T-cell lymphoma may be beneficial.

Abbreviations

CHOP	A chemotherapy protocol that includes cyclophosphamide, doxorubicin, vincristine, and prednisone
CLOP	A chemotherapy protocol that includes cyclophosphamide, lomustine, vincristine, and prednisone
PFS	Progression-free survival
OST	Overall survival time
L-CHOP	A chemotherapy protocol that includes L-asparaginase, cyclophosphamide, doxorubicin, vincristine, and prednisone
MOPP	A chemotherapy protocol that includes mechlorethamine, vincristine, procarbazine and prednisone
LOPP	A chemotherapy protocol that includes lomustine, vincristine, procarbazine and prednisolone
CEOP	A chemotherapy protocol that includes cyclophosphamide, vincristine, epirubicin and prednisolone
LEOP	A chemotherapy protocol that includes cyclophosphamide, vincristine, epirubicin and lomustine
VELCAP-TSC	A chemotherapy protocol that includes vincristine, L-asparaginase, cyclophosphamide, lomustine, doxorubicin, prednisolone, procarbazine and mechlorethamine
TZL	T-zone lymphoma
WHO	World Health Organization
CBC	Complete blood count
HCT	Hematocrit
PLT	Platelet
AEs	Adverse events
VCOG	Veterinary Cooperative Oncology Group
CR	Complete remission
PR	Partial remission
PD	Progressive disease
SD	Stable disease
CTCAE	Common terminology criteria for adverse events
ALT	Alanine aminotransferase
DFI	Disease free interval

Acknowledgements
Not applicable.

Authors' contributions
AE, GD and EY were the clinicians treating the dogs in the study. EH and MSK performed statistical analysis for the study. AD provided internal medicine insight. CAG helped designing the CLOP protocol. All authors read and approved the final manuscript.

Funding
Not applicable.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Israeli council for experiments in animal subjects PET-2023–21-NE.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 18 July 2024 Accepted: 11 September 2024
Published online: 27 September 2024

References

1. Vail D, Thamm D, Liptak J. Hematopoietic tumors. In: Vail DM, Pinkerton M, Young KM, editors. Withrow & MacEwen's Small Animal Clinical Oncology. 6th ed. St Louis, MO: Elsevier; 2020. p. 688.
2. Garrett LD, Thamm DH, Chun R, Dudley R, Vail DM. Evaluation of a 6-month chemotherapy protocol with no maintenance therapy for dogs with lymphoma. J Vet Intern Med. 2002;16:704–9.
3. Burton JH, Garrett-Mayer E, Thamm DH. Evaluation of a 15-week CHOP protocol for the treatment of canine multicentric lymphoma. Vet Comp Oncol. 2013;11(4):306–15.
4. Sorenmo K, Overley B, Krick E, Ferrara T, LaBlanc A, Shofer F. Outcome and toxicity associated with a dose-intensified, maintenance free CHOP-based chemotherapy protocol in canine lymphoma: 130 cases. Vet Comp Oncol. 2010;8(3):196–208.
5. Hosoya K, Kisseberth WC, Lord LK, et al. Comparison of COAP and UW-19 protocols for dogs with multicentric lymphoma. J Vet Intern Med. 2007;21(6):1355–63.
6. Chun R, Garrett LD, Vail DM. Evaluation of a high-dose chemotherapy protocol with no maintenance therapy for dogs with lymphoma. J Vet Intern Med. 2000;14:120–4.
7. Curran K, Thamm DH. Retrospective analysis for treatment of naïve canine multicentric lymphoma with a 15-week, maintenance-free CHOP protocol. Vet Comp Oncol. 2016;14(1):147–55.
8. Rebhun RB, Kent MS, Borrofka SAEB, Frazier S, Skorupski K, Rodriguez CO. CHOP chemotherapy for the treatment of canine multicentric T-cell lymphoma. Vet Comp Oncol. 2010;9(1):38–44.
9. Angelo G, Cronin K, Keys D. Comparison of combination l-asparaginase plus CHOP or modified MOPP treatment protocols in dogs with multi-centric T-cell or hypercalcaemic lymphoma. J Small Anim Pract. 2019;60:430–7.
10. Morgan E, O'Connell K, Thomson M, Griffin A. Canine T cell lymphoma treated with lomustine, vincristine, procarbazine, and prednisolone chemotherapy in 35 dogs. Vet Comp Oncol. 2018;16:622–9.
11. Brown PM, Tzannes S, Nguyen S, White J, Langova V. LOPP chemotherapy as a first-line treatment for dogs with T-cell lymphoma. Vet Comp Oncol. 2018;16:108–13.
12. Blaxill J, Buzzacott P, Finlay J. Prognostic indicators for naïve canine non-indolent T-cell lymphoma treated with combination lomustine, vincristine, procarbazine and prednisolone chemotherapy. Vet Comp Oncol. 2022;20:215–26.
13. Brodsky EM, Maudlin GN, Lachowicz JL, Post GS. Asparaginase and MOPP treatment of dogs with lymphoma. J Vet Intern Med. 2009;23:578–84.
14. Elliott J, Baines S. A retrospective study of multi-agent chemotherapy including either cyclophosphamide or lomustine as initial therapy for canine high-grade T-cell lymphoma (2011–2017). Aust Vet J. 2019;97:308–15.
15. Goodman IH, Moore AS, Frimberger AE. Treatment of canine non-indolent T cell lymphoma using the VELCAP-TSC protocol: a retrospective evaluation of 70 dogs (2003–2013). Vet J. 2016;211:39–44.
16. Saba CF, Clifford C, Burgess K, et al. Rabacfosadine for naïve canine intermediate to large cell lymphoma: Efficacy and adverse event profile across three prospective clinical trials. Vet Comp Oncol. 2020;18:763–9.
17. Musser ML, Clifford CA, Bergman PJ, et al. Randomised trial evaluating chemotherapy alone or chemotherapy and a novel monoclonal antibody for canine T-cell lymphoma: A multicentre US study. Vet rec open. 2022;9(1):e49.
18. Moore AS. Treatment of T cell lymphoma in dogs. Vet Rec. 2016;179(11):277.
19. Zandvliet M, Teske E. Mechanisms of drug resistance in veterinary oncology - a review with an emphasis on canine lymphoma. Vet Sci. 2015;2:150–84.

20. Klimecki WT, Futscher BW, Grogan TM, Dalton WS. P-glycoprotein expression and function in circulating blood cells from normal volunteers. Blood. 1994;83(9):2451–8.
21. Culmsee K, Gruber AD, Von Samson-Himmelstjerna G, Nolte I. (2004) Quantification of MDR-1 gene expression in canine tissues by real-time reverse transcription quantitative polymerase chain reaction. Res Vet Sci. 2004;77(3):223–9.
22. Beaver LM, Strottner G, Klein MK. Response rate after administration of a single dose of doxorubicin in dogs with B-cell or T-cell lymphoma: 41 cases (2006–2008). J Am Vet Med Assoc. 2010;237(9):1052–5.
23. Sampaio F, Marrinhas C, Oliveira LF, et al. Detection of Lymphoid Markers (CD3 and PAX5) for immunophenotyping in dogs and cats: comparison of stained cytology slides and matched cell blocks. Vet Sci. 2023;10:157–72.
24. Valli VE, San Myint M, Barthel A, et al. Classification of canine malignant lymphomas according to the World Health Organization criteria. Vet Pathol. 2011;48(1):198–211.
25. Graff EC, Spangler EA, Smith A, Denhere M, Brauss M. Hematologic findings predictive of bone marrow disease in dogs with multicentric large-cell lymphoma. Vet Clin Pathol. 2014;43(4):505–12.
26. Vail D, Michels G, Khanna C, Selting KA, London CA. Response evaluation criteria for peripheral nodal lymphoma in dogs (v1.0) - a Veterinary Cooperative Oncology Group (VCOG) consensus document. Vet Comp Oncol. 2010;8(1):28–37.
27. LeBlanc AK, Atherton M, Bentley RT, et al. Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) following investigational therapy in dogs and cats. Vet Comp Oncol. 2021;19(2):311–52.
28. Siedlecki CT, Kass PH, Jakubiak MJ, Dank G, Lyons J, Kent MS. Evaluation of an actinomycin-D-containing combination chemotherapy protocol with extended maintenance therapy for canine lymphoma. Can Vet J. 2006;47:52–9.
29. Fournier Q, Serra JC, Handel I, Lawrence J. Impact of pretreatment neutrophil count on chemotherapy administration and toxicity in dogs with lymphoma treated with CHOP chemotherapy. J Vet Intern Med. 2018;32(1):384–93.
30. Purzycka K, Peters LM, Desmas I, Davies O, Chang YM, Lara-Garcia A. Clinicopathological characteristics and prognostic factors for canine multicentric non-indolent T-cell lymphoma: 107 cases. Vet Comp Oncol. 2020;18(4):656–63.
31. Jankowska U, Jagielski D, Czopowicz M, Sapierzynski R. The animal dependent risk factors in canine T-cell lymphomas. Vet Comp Oncol. 2017;15:307–14.
32. Bennett PF, Taylor R, Williamson P. Demographic risk factors for lymphoma in Australian dogs: 6201 cases. J Vet Intern Med. 2018;32:2054–60.
33. Starrak GS, Berry CR, Page RL, Johnson JL, Thrall DE. Correlation between thoracic radiographic changes and remission/survival duration in 270 dogs with lymphosarcoma. Vet Radiol Ultrasound. 1997;38(6):411–8.
34. Rosenberg MP, Matus RE, Patnaik AK. Prognostic factors in dogs with lymphoma and associated hypercalcemia. J Vet Intern Med. 1991;5(5):268–71.
35. Marconato L, Stefanello D, Valenti P, et al. Predictors of long-term survival in dogs with high-grade multicentric lymphoma. J Am Vet Med Assoc. 2011;238(4):480–5.
36. Kaiser CI, Fidel JL, Roos M, Kaser-Hotz B. Reevaluation of the University of Wisconsin 2-year protocol for treating canine lymphosarcoma. J Am Anim Hosp Assoc. 2007;43(2):85–92.
37. Nerschbach V, Eberle N, Joetzke AE, et al. Splenic and hepatic ultrasound and cytology in canine lymphoma: effects of findings on stage migration and assessment of prognosis. Vet Comp Oncol. 2016;14(S1):82–94.

Publisher's Note