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### Evaluation of the results of a L-asparaginase-based continuous chemotherapy protocol versus a short doxorubicin-based induction chemotherapy protocol in dogs with malignant lymphoma

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# EVALUATION OF THE RESULTS OF A L-ASPARAGINASE-BASED CONTINUOUS CHEMOTHERAPY PROTOCOL VERSUS A SHORT DOXORUBICIN-BASED INDUCTION CHEMOTHERAPY PROTOCOL IN DOGS WITH MALIGNANT LYMPHOMA

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## Original Papers

### SUMMARY

The results of an L-asparaginase-based continuous chemotherapy protocol ( $n = 52$ ) versus a short doxorubicin-based induction chemotherapy protocol ( $n = 65$ ) were evaluated in 117 dogs with malignant lymphoma. There were no differences between the two groups in patient characteristics or incidence of protocol-related toxicity. Complete remission was induced in 71.2% of the dogs treated with the L-asparaginase protocol and in 67.7% of the dogs treated with the doxorubicin-plus protocol. The calculated Kaplan-Meier one- and two-year survival fractions in the L-asparaginase group were 48% and 26%, and in the doxorubicin-plus group 35%, and 22%, respectively. Differences in remission and survival between the two treatment groups were not significant. A multivariate Cox proportional hazards survival analysis revealed that elevated pretreatment plasma creatinine concentration and prior treatment with prednisolone were associated with shorter survival times. An elevated pretreatment plasma creatinine concentration and total leucocyte count were associated with a decrease in the disease-free period. Differences in efficacy and toxicity between the two protocols were not significant. There is no apparent advantage in using the continuous L-asparaginase protocol, and the shorter doxorubicin-plus protocol is less expensive and less time consuming.

**Keywords:** Malignant lymphoma, non-Hodgkin's lymphoma, chemotherapy, L-asparaginase, doxorubicin, dog.

### INTRODUCTION

Multicyclic chemotherapy protocols have been most successful in the treatment of malignant lymphoma in the dog. The mainstays of these protocols are doxorubicin, cyclophosphamide, L-asparaginase, and vincristine. Reported complete remission rates of the best protocols vary between 65% and 84% and estimated one-year survival fractions between 27% and 42% (2,4,7,14,15). The choice among different treatment protocols may be influenced by efficacy (i.e. response rate, duration of remission, and survival time), treatment-related toxicity, and cost in both time and money. In addition, the physical condition of the patient may be important. Although the toxicity of an aggressive course of induction

chemotherapy is well accepted in human medicine as long as it induces long periods of remission, such induction protocols are usually not acceptable to owners of veterinary patients. The induction chemotherapy protocols used in dogs are thus less intense than those used in human medicine through limitation of the dose or prolongation of the dosage interval. Important advantages of an induction chemotherapy protocol without a maintenance phase are that it reduces costs and time for the owner and reduces the number of visits to the clinic and therefore the stress to the patient.

In this study, the results of two multicyclic chemotherapy protocols based on either L-asparaginase or doxorubicin were evaluated. In the L-asparaginase protocol the induction phase was followed by a maintenance phase. The doxorubicin-based protocol consisted of an induction phase only. The objectives were to determine whether the two protocols are equally effective in the treatment of canine malignant lymphoma and to identify prognostic factors and protocol-related toxicity.

### MATERIALS AND METHODS

#### Animals

The medical records of dogs in which malignant lymphoma was diagnosed and subsequently treated by chemotherapy between January 1992 and June 1998 were reviewed. Only the medical records of the dogs treated either with the L-asparaginase protocol (Table 1) or the doxorubicin-plus protocol (Table 2) were selected for evaluation. Breed, sex, age at

Table 1. L-asparaginase chemotherapy protocol.

Day 1	L-asparaginase	400 IU/kg IM
Day 8	L-asparaginase	400 IU/kg IM
Day 15	Vincristine	0.5-0.7 mg/m <sup>2</sup> IV
	Prednisolone	2 mg/kg PO daily
Day 22	Cyclophosphamide	300 mg/m <sup>2</sup> PO
	Prednisolone	1.5 mg/kg PO daily
	L-asparaginase	400 IU/kg IM
Day 29	Vincristine	0.5-0.7 mg/m <sup>2</sup> IV
	Prednisolone	1 mg/kg PO daily
Day 36	Doxorubicin	30 mg/m <sup>2</sup> IV
	Prednisolone	0.5 mg/kg PO daily
Day 50	Vincristine	0.5-0.7 mg/m <sup>2</sup> IV
	Prednisolone	0.25 mg/kg PO daily
Day 57	Chlorambucil	1.4 mg/kg PO
	L-asparaginase	400 IU/kg IM
	Stop prednisolone	
Day 64	Vincristine	0.5-0.7 mg/m <sup>2</sup> IV
Day 71	Doxorubicin	30 mg/m <sup>2</sup> IV
	L-asparaginase	400 IU/kg IM
Day 78	L-asparaginase	400 IU/kg IM

Continue L-asparaginase treatments every 14 days.  
If relapse occurs, choose second-line treatment.

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Table 2. Doxorubicin-plus chemotherapy protocol.

Day 1	L-asparaginase	400 IU/kg IM
Day 8	Dexamethasone	10 mg/m <sup>2</sup> IV
	Doxorubicin	30 mg/m <sup>2</sup> IV
Day 22	Dexamethasone	10 mg/m <sup>2</sup> IV
	Doxorubicin	30 mg/m <sup>2</sup> IV
Day 23	Prednisolone	50mg/m <sup>2</sup> PO daily (decrease dose by 1/4 weekly)
Day 33/34	Chlorambucil	25 mg/m <sup>2</sup> PO divided over 2 days
Day 43	Doxorubicine	30 mg/m <sup>2</sup> IV
Day 57	L-asparaginase	400 IU/kg IM
Day 60	Cyclophosphamide	200-250 mg/m <sup>2</sup> PO
Day 71	Doxorubicine	30 mg/m <sup>2</sup> IV
Day 80/81	Chlorambucil	25 mg/m <sup>2</sup> PO divided over 2 days
Day 92	Doxorubicine	30 mg/m <sup>2</sup> IV
Day 113	Cyclophosphamide	200-250 mg/m <sup>2</sup> PO
Day 127	Evaluation:	if in CR, then give hydroxyurea 500mg/m <sup>2</sup> PO daily for 3 weeks
Day 148	If CR:	stop therapy

diagnosis, body weight, haematocrit, leucocyte count, plasma concentration of calcium, creatinine, and bile acids, and the stage and whether there had been pretreatment with prednisolone were recorded and evaluated as possible prognostic indicators. In all cases the diagnosis was made by cytological examination of a fine-needle aspirate or histological examination of a tissue specimen.

#### Treatment

After clinical evaluation and staging (WHO) (17) the dogs were allocated to receive chemotherapy treatment according either to the L-asparaginase or the doxorubicin-plus protocol. The induction phase of the L-asparaginase protocol was followed by a maintenance phase of biweekly intramuscular injections of L-asparaginase in those animals in which there was complete remission. If the induction phase of the L-asparaginase protocol or the doxorubicin-plus protocol resulted in only a partial response or in progressive disease, second-line therapy was proposed. This consisted of vincristine, cyclophosphamide, and prednisolone plus either doxorubicin or L-asparaginase, depending on the first protocol used. The same second-line therapy was proposed for dogs that relapsed during the maintenance phase of the first protocol, or during the treatment-free period after the induction phase of the doxorubicin-plus protocol. In all cases the total cumulative dose of doxorubicin was kept below 200 mg/m<sup>2</sup>. Because of the retrospective nature of this study, the patients were not assigned to one or the other treatment by a randomized procedure. Instead, the choice was usually made by the owner based on financial or other personal reasons.

#### Response criteria

All dogs were evaluated at each treatment. Disappearance of all measurable tumour was considered to be a complete remission. A partial remission was defined as a decrease in total tumour volume by greater than 50%, provided that no new lesions had developed and no lesions had progressed. A decrease of less than 50% in tumour volume and an increase of no more than 25% in any of the measurable lesions was considered to be stable disease. Progressive disease was defined as a 25% or greater increase in the size of one or more measurable lesions or the appearance of new lesions. The incidence of protocol-related side-effects such as vomiting, diarrhea, bone marrow depression, alopecia, and tu-

mour lysis syndrome was recorded. Vomiting and diarrhea were categorized on the basis of their frequency and severity. Vomiting was categorized as being absent, only represented by anorexia, transient, or continuous (vomiting more than 1-2 times per day for more than 2 days). Diarrhea was categorized as absent, lasting up to 2 days, or lasting more than 2 days. The response rate was defined as the percentage of dogs in which complete remission occurred. Survival time was calculated on the basis of time of entry into the study to death or to the date on which the dog was last known to be alive, including all dogs, and counting only deaths due to lymphoma as events. For duration of response in animals undergoing complete remission, the time to relapse was defined as the interval between entry into the study and relapse, or the date on which the dog was last known to be free of disease, counting only relapses as events.

#### Cytological classification

All available cytology and histology slides were re-evaluated and classified according to the Kiel classification (5) by one of the authors (E.T.). A detailed classification based on immunophenotyping was not possible due to lack of tissue material in several dogs.

Table 3. Patients' characteristics.

Patients' characteristics	L-asparaginase n = 52	Doxorubicin-plus n = 65	Total n = 117
Purebred dogs	48 (27 breeds)	55 (33 breeds)	103
Mongrels	4	10	14
Sex			
male	20	22	42
castrated male	6	8	14
female	12	13	25
castrated female	14	22	36
Age (mean, range)	7.2 (1.2-13.5)	7.6 (1.5-17.2)	
≤ 5 years	16	23	39
6-9 years	28	29	57
≥ 10 years	8	13	21
Body weight			
< 20 kg	9	15	24
20-30 kg	35	33	68
≥ 40 kg	8	17	25
Type of tumour			
multi centric	41	49	90
alimentary	0	6	6
thymus	2	2	4
skin	7	4	11
leukemia	1	0	1
others	1	4	5
Stage of tumour			
localized	6	8	14
systemic	37	32	69
spleen, liver	3	4	7
bone marrow, leukemia	6	21	27
Kiel classification			
low-grade malignancy	6	6	12
high-grade malignancy	39	49	88
missing	7	10	17



### Statistics

Statistical analysis of data was performed using the SPSS (8) and EGRET (1) statistical packages. Differences between the two treatment groups were evaluated by the Chi-square test for ordinal data or ratio data and by the two-tailed non-paired Student's *t*-test or one-way parametric analysis of variance for interval data. Survival curves were drawn with the Kaplan-Meier method. The parameters sex, age, body weight, plasma calcium and creatinine concentrations, stage, pretreatment with prednisolone, and protocol deviations were introduced in a univariate model to determine the influence of these parameters on the calculated disease-free period and the calculated survival. Univariate tests for comparison of groups of survival data were made with the log-rank test and with a proportional hazard logistic regression model.

For testing the influence of different variables on the survival data a multivariate Cox's proportional hazards model regression analysis of survival was performed with a forward stepwise selection. Only those parameters that have been reported to be prognostic indicators or had  $P < 0.20$  in the univariate analysis and only data from those dogs for which all parameters were available were included in the multivariate analysis. In these analyses the animals were stratified according to their treatment groups.  $P < 0.05$  was considered significant.

## RESULTS

### Patient characteristics

Patient characteristics are listed in table 3. Fifty-two dogs were treated with the L-asparaginase protocol and 65 dogs with the doxorubicin-plus protocol. Differences between the two groups with regard to breed, sex, age, body weight, and type, stage and cytological grading of the lymphoma were not significant.

### Results of treatment

Treatment results are listed in table 4. During induction chemotherapy the L-asparaginase protocol induced complete remission in 37 (71.2%) dogs, partial remission in 11 (21.2%) dogs, stable disease in 1 (1.9%) dog, and progressive disease in 3 (5.8%) dogs. Among the 48 dogs in which there was either complete or partial remission, relapse occurred in 38 dogs. Thirty-five of the dogs that had a relapse received second-line therapy. The doxorubicin-plus protocol induced complete remission in 44 (67.7%) dogs, partial remission in 10 (15.4%), stable disease in 1 (1.5%), and progressive disease in 10 (15.4%). Among the 54 dogs in which there was either complete or partial remission, relapse occurred in 42 dogs. Thirty of the dogs that had a relapse received second-line therapy. Differences in response and treatment-related toxicity between the two protocols were not significant. Five dogs were hospitalized during induction chemotherapy because of protocol-related toxicity. In two of these fluids had to be administered because of dehydration due to vomiting and diarrhea. In three other dogs tumour lysis syndrome was diagnosed. Adequate information about the haematocrit, total leucocyte count, and thrombocyte count to determine the incidence and severity of bone marrow depression during the induction period was missing in 42 of the 117 cases. In two dogs localized cellulitis and in two dogs severe localized tissue necrosis developed in the front leg around the saphenous vein due to perivascular leakage of doxorubicin.

The median disease-free period was 216 and 203 days and the median overall survival time was 236 and 200 days for

Table 4. Response to treatment and treatment related toxicity in dogs with malignant lymphoma.

Patient response	L-asparaginase n = 52	Doxorubicin-plus n = 65	Total n = 117
<b>Response</b>			
progressive disease	3	10	13
stable disease	1	1	2
partial remission	11	10	21
complete remission	37	44	81
<b>Vomiting</b>			
missing data <sup>1</sup>	2		2
none	29	36	65
anorexia	3	6	9
transient vomiting	14	13	27
constant vomiting	4	10	14
<b>Diarrhea</b>			
missing data <sup>1</sup>	2		2
none	30	40	70
≤ 2 days	7	11	18
≥ 2 days	13	14	27
<b>Tumour lysis</b>	2	1	3
<b>Alopecia</b>		1	1
<b>Protocol deviations</b>	22	20	42

<sup>1</sup> In 2 dogs vomiting and diarrhea was already present before chemotherapy started and therefore protocol-related toxicity could not be determined.

the L-asparaginase and doxorubicin-plus groups, respectively. The long-term follow-up results are illustrated by curves for the estimated disease-free period (Figure 1) and survival (Figure 2). The one-year estimated disease-free fraction in the L-asparaginase group was 31.9% (95% confidence interval (CI) 17.3 - 47.4%), while the estimated one-year and two-year survival was 48% (95% CI, 33.7 - 61.2%) and 26% (95% CI, 14.2 - 39.7%), respectively. In the doxorubicin-plus group the one-year estimated disease-free fraction was 29.2% (95% CI, 15.8 - 43.9%) and the estimated one-year and two-year survival was 35% (95% CI, 22.7 -

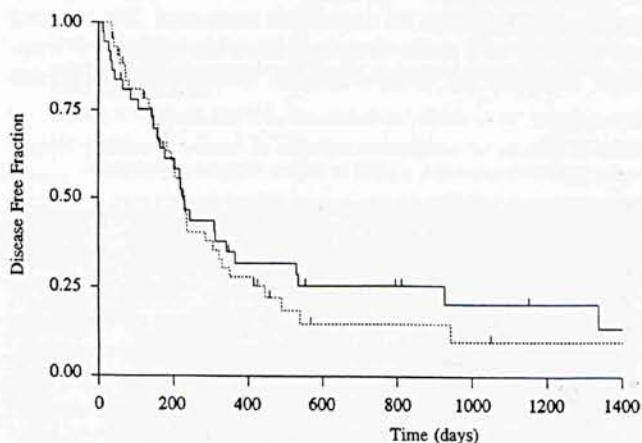


Figure 1. Curve of the estimated disease-free interval in dogs with malignant lymphoma treated with the L-asparaginase (n = 52) and doxorubicin-plus protocol (n = 65).

L-asparaginase ———  
Doxorubicin-plus - - - -



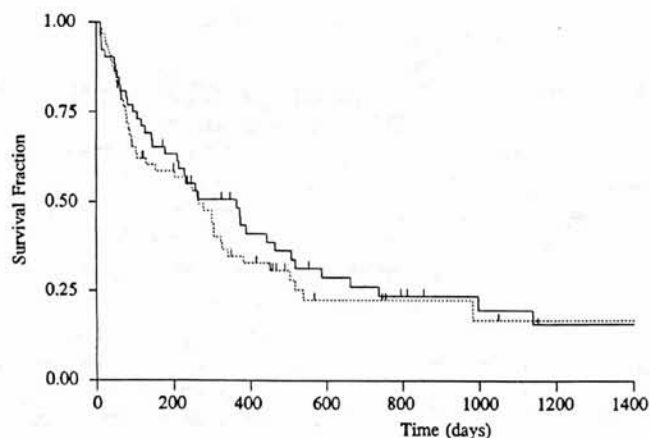


Figure 2. Survival curve for dogs with malignant lymphoma treated by the L-asparaginase protocol (n = 52) and doxorubicin-plus protocol (n = 65).

L-asparaginase ————  
Doxorubicin-plus - - - - -

46.9%) and 22% (95% CI, 11.7 - 35.0%), respectively. Differences between the two treatment groups in disease-free period and survival were not significant.

The parameters that were included in the multivariate analysis as predictors for a relapse were sex, age, body weight, pretreatment plasma calcium and creatinine concentrations, the occurrence of vomiting during induction chemotherapy, and stage, protocol deviations, and pretreatment total leucocyte count. An elevated pretreatment plasma creatinine concentration and an increase in total leucocyte count were associated with a decrease in the length of the disease-free period (Table 5). The parameters that were included as predictors for death in the multivariate analysis were sex, pretreatment calcium and creatinine concentrations, age, body weight, prior treatment with prednisolone, stage, and protocol deviations. Elevated pretreatment plasma creatinine concentration and prior treatment with prednisolone were associated with shorter survival times. The occurrence of protocol deviations was associated with increased survival times (Table 6).

## DISCUSSION

In this study two different protocols for the treatment of malignant lymphoma in dogs were evaluated. The toxicity and efficacy of a multi-drug induction chemotherapy treatment followed by L-asparaginase maintenance treatment

Table 5. Results of multivariate analysis of factors predicting relapse (using disease-free period, n = 59) in canine malignant lymphoma.

Patients' characteristics	P <sup>1</sup> Hazard ratio	95% CI <sup>2</sup>
Plasma creatinine concentration <sup>3</sup>		
normal		
mild evaluation	0.001	20.57 3.37-125.7
severe evaluation	0.252	2.41 0.53-10.9
Pretreatment total leucocyte count	0.054	1.03 1.00-1.07

<sup>1</sup> P was calculated for each factored variable, the reference being dogs with normal plasma creatinine concentration and a total leucocyte count of 3 giga/l prior to treatment.

<sup>2</sup> 95% CI = 95% Confidence Interval.

<sup>3</sup> Categories of plasma creatinine concentration (CREAT) in  $\mu\text{mol/l}$  related to body weight (BW in kg): normal ( $< 75 + 1.2 \cdot \text{BW}$ ), mild increase (increase  $75 + 1.2 \cdot \text{BW} < \text{CREAT} < 110 + 1.2 \cdot \text{BW}$ ), severe increase ( $\text{CREAT} > 110 + 1.2 \cdot \text{BW}$ ).

were compared with those of a short course of doxorubicin-based induction chemotherapy without maintenance treatment. The potential advantage of the latter is a reduction in both costs and the number of appointments for treatment.

Although assignment to the treatment groups was not randomized, the choice of one or the other treatment protocol was always made by the owner and not the clinician. This choice was only based on the cost and number of treatment appointments required, not on the condition of the patient. No differences in patient characteristics between the two treatment groups were found to be significant.

Toxicity is one of the main problems of chemotherapy protocols and often of main concern to the owner. No significant difference in toxicity could be found between the two types of treatment in this study. Anorexia, vomiting, and diarrhea have been reported to occur in up to 40% of the patients treated on protocols comparable to those used in this study (4,7,13). Although the incidence of toxicity in this study was comparable, the severity of the side-effects was difficult to compare. Definitions of toxicity are not always clear and may differ. In some studies (7,13,16) 10-29% of the treated dogs had such severe side-effects that hospitalization was necessary. In the present study only five dogs were hospitalized. Three of these dogs had tumour lysis syndrome, which is caused by the acute lysis of tumour cells following chemotherapy and the subsequent release of intracellular products and their metabolites (11). Other reported side-effects include bone marrow depression, congestive cardiomyopathy, and tissue necrosis due to extravasation of chemotherapeutics (9).

Table 6. Results of multivariate analysis of factors predicting death (using survival time, n = 81) in canine malignant lymphoma.

Patients' characteristics	P <sup>1</sup> Hazard ratio	95% CI <sup>2</sup>
Plasma creatinine concentration <sup>3</sup>		
normal		
mild evaluation	<0.001	12.11 3.40-43.16
severe evaluation	0.003	5.84 1.86-18.34
Protocol deviations		
no		
yes	0.019	0.5 0.28-0.89
Prior treatment with prednisolone		
no		
yes	0.028	2.002 1.08-3.71

<sup>1</sup> P was calculated for each factored variable, the reference being dogs with normal plasma creatinine concentration, no protocol deviations, and no prior treatment with prednisolone.

<sup>2</sup> 95% CI = 95% Confidence Interval.

<sup>3</sup> Categories of plasma creatinine concentration (CREAT) in  $\mu\text{mol/l}$  related to body weight (BW in kg): normal ( $< 75 + 1.2 \cdot \text{BW}$ ), mild increase (increase  $75 + 1.2 \cdot \text{BW} < \text{CREAT} < 110 + 1.2 \cdot \text{BW}$ ), severe increase ( $\text{CREAT} > 110 + 1.2 \cdot \text{BW}$ ).

The difference in the percentage of patients achieving complete remission after the induction phase of the two treatment protocols was not significant. A partial response was not considered to be a success in this study and second-line therapy was given to patients in which it occurred. The percentage of patients that achieved complete remission, 71% and 68% in the L-asparaginase and doxorubicin-plus groups, respectively, is comparable to reported remission rates of 65-84% (2,4,7,14,15). The differences in disease-free period and overall survival between the two treatment groups were also not significant. Survival periods are often difficult to



compare between different studies. Often-used parameters such as median disease-free period and median survival do not take into account the advantages of censoring. Therefore, those parameters that are based on life-table estimates methods, such as the Kaplan-Meier analysis, are preferable (10). However, to facilitate comparison of the results of the present study with the results of other studies, both types of parameters are reported in this study. The estimated one-year disease-free fractions and the estimated one- and two-year survival fractions of 31%, 48%, and 26% for the L-asparaginase group and 29%, 45%, and 22% for the doxorubicin-plus group, respectively, are among the highest and longest reported (Table 7) (2,4,7,14).

It is essential to identify independent prognostic factors by means of multivariate analysis in order to advise owners about the treatment of malignant lymphoma. Until now, however, prognostic factors have not yet been used in the formulation and modification of treatment protocols in animals as they have been used in humans. Sex (4,6), age (7), body weight (16), clinical stage (15) or substage (4,7,16), histological classification (Kiel classification, Working Formulation) (2, 15), immunophenotype (2, 15), pretreatment plasma calcium concentrations (16), and prior treatment with prednisolone (12) have been reported to be valuable indicators for predicting relapse, survival, or both. In the present study, an elevated pretreatment plasma creatinine concentration and leucocytosis were associated with a decreased duration of the disease-free period. In contrast to earlier findings (15), the Kiel classification was not found to be an independent prognostic variable in this study. This is most likely due to the low number of dogs in the low-grade malignancy group and the lack of correction for immunophenotype in this study.

An elevated pretreatment plasma creatinine concentration and prior treatment with prednisolone were associated with shorter survival times in this study. Pretreatment with prednisolone has also been reported to decrease the duration of remission (12). This implies that veterinary practitioners should refrain from administering glucocorticoids to dogs with malignant lymphoma before chemotherapy is started by a veterinary oncologist. Protocol deviations, necessitated by protocol-related toxicity or owner non-compliance, did not shorten the survival time. Response to treatment has been reported to be an important independent prognostic indicator affecting survival (16). However, response to treatment is not a pretreatment prognostic variable and therefore cannot be used in decision-making beforehand. For this reason, and because the

presence of such an additional and powerful variable in the multivariate analysis would affect the ability to discover other predictive variables, the factor response to treatment was not included in the multivariate analysis in this study.

The question remains whether continuous chemotherapy protocols as opposed to short induction protocols are really necessary. Not many veterinary reports address this issue. Hahn *et al.* (3) compared short-term doxorubicin therapy with long-term COP (cyclophosphamide, vincristine, prednisone) and found no difference between them in the time to first remission or in the survival time, which is in agreement with our findings. The doxorubicin-plus protocol in the study reported here is 148 days long, while other protocols are much longer or have a continuous maintenance phase after the induction phase. The Kaplan-Meier curves for the calculated disease-free fraction in this study diverged (Figure 1) 250 days after the start of treatment. This suggests that the continuation of chemotherapy in the L-asparaginase group had a positive effect, but the difference was not significant even after 250 days of treatment, although the low number of patients still in remission could account for this. An advantage of stopping chemotherapy in the doxorubicin-plus group is that patients that are cured by the induction protocol will not receive unnecessary additional chemotherapy. In the patients that received doxorubicin-plus treatment and relapsed a second remission was easily induced and maintained, leading to survival times similar to those in the L-asparaginase group. Since the differences in efficacy and toxicity between the L-asparaginase maintenance protocol and the short course of doxorubicin-plus induction treatment were not significant, there are no medical reasons to prefer the L-asparaginase maintenance protocol in the treatment of canine malignant lymphoma. The short doxorubicin-plus protocol, offers a substantial financial benefit to the owner, due to lower costs for medication and less frequent visits to the clinic and it also requires less time and effort on the part of the clinician.

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Table 7. Results of different chemotherapy protocols in dogs with malignant lymphoma.

Induction Protocol <sup>1</sup>	Maintenance	n	Complete remission (%)	1-year remission (%)	1-year survival (%)	2-year survival (%)	Ref
VCR,CTX,DOX,ASP,PRED <sup>2</sup>	NO	112	73	NR	42	28	2
VCR,CTX,DOX,ASP,PRED	YES	138	77	NR	42	NR	15
VCR,CTX,DOX,ASP,PRED	YES	68	76	40	27	13	7
VCR,CTX,DOX,ASP,MTX,PRED	YES	55	84	42	52	24	4
<b>Present study</b>							
VCR,CTX,DOX,ASP,PRED	YES	52	71	32	48	26	
VCR,CTX,DOX,ASP,PRED	NO	65	68	29	45	22	

<sup>1</sup> DOX: doxorubicin; VCR: vincristine; CTX: cyclophosphamide; PRED: prednisolone; MTX: methotrexate; ASP: L-asparaginase, NR: not reported.

<sup>2</sup> Induction protocol lasts 36 weeks.



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