國立臺灣大學獸醫專業學院臨床動物醫學研究所

碩士論文

Graduate Institute of Veterinary Clinical Sciences

School of Veterinary Medicine

National Taiwan University

Master Thesis

比較犬多中心型淋巴瘤 15 週與 25 週化療療程之成效與副作用

Comparison of Efficacy and Toxicity of 15-Week and 25-Week Chemotherapy Protocol in Canine Multicentric Lymphoma

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中華民國 107 年 7 月

July, 2018

doi:10.6342/NTU201801637

國立臺灣大學碩士學位論文
口試委員會審定書

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本論文係 高嘉蓮君（學號R04643005）在國立臺灣大學臨床動物醫學研究所完成之碩士學位論文，於民國107年7月3日承下列考試委員審查通過及口試及格，特此證明

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doi:10.6342/NTU201801637

誌謝

在腫瘤科轉眼間也待滿了三年，除了學識上的不間斷的經驗累積及學習，很重要的是感謝有一群能讓我順利完成這篇論文的老師和夥伴們。首先謝謝李繼忠老師給予的訓練和教導，在需要的時候總會在背後提供幫助，並支持我為這篇論文進行後續的延伸工作。已畢業的學長姐怡蓓、淳翔、怡甄、佩晴、宜君，在我剛投入腫瘤學習的階段，都很認真地帶領我認識這個領域，一步步看著、學著作為榜樣的他們，也讓我能夠逐漸熟悉各種臨床知識和技能。

再來要非常感謝影響我最深的兩位學長，柏穎和育廷，兩位如親哥哥般的存在在研究所整整三年間，都在我身邊指導和支援，也始終是我學習的榜樣。即使到了獨立看診的第三年，不管是臨床上、研究上或生活上，都仍然得到了他們很多的幫助與援救，真心感謝有他們在身邊，能有這樣的緣分對我來說是難能可貴而何其幸運的。另外，晶玫、君婷、映華、涵勻、欣怡、韋葶、緣緣、嘉心這幾位學妹，也都是我極為重要的夥伴們，大家在腫瘤科日漸培養出的臨床能力，在門診時成了不可缺少的助力。感謝可靠的她們，以及醫院曾給予我幫助的所有醫師和大五排診學生，讓我能夠在論文寫作的期間無後顧之憂，即使門診論文兩頭燒，還能夠如期完成進度。資料統計上，要感謝統計中心張嘉鴻學長，花了很多時間與我討論和修正統計的方向及結果，研究資料數量之龐大，也曾讓學長煞費不少苦心，很慶幸在我不熟悉的統計領域上能夠得到學長許多專業幫助。此外也感謝醫院病歷室的人員，因為回溯病歷的關係，時常麻煩了他們。

最後感謝爸爸媽媽及所有家人，在把家裡當飯店的無數個日子以來，仍舊不嫌棄也尊重並任由我走想走的路。

doi:10.6342/NTU201801637

中文摘要

犬多中心型淋巴瘤為犬隻最常見之淋巴增生性疾病。多種藥物組合之化學治療因有機會達到高治療反應率、持續之疾病消退期、相對較長之存活時間，故成為主流治療方式。其中，改良之威斯康辛麥迪遜大學無維持期療程（即 25 週療程）已被廣泛使用，搭配藥物包含 vincristine、cyclophosphamide、doxorubicin 和類固醇（簡稱 CHOP）。雖然多數病患對治療反應良好，但最終仍會因疾病復發而死亡。為了增進治療效果、縮短療程長度和花費，將劑量更加密集給予以改良療程的方式開始被提出，包括化療藥物的同時給予、縮短藥物給予間隔或增加藥物劑量等。15 週 CHOP 療程即因此被提出，初步研究顯示效果和過往相當且毒性可耐受 [1]。本研究的目的為更深入地針對此 15 週 CHOP 療程和上述 25 週療程進行成效與毒性比較，而此兩種療程也是國立臺灣大學生物資源暨農學院附設動物醫院最常使用之兩種療程。

本研究以回溯性方法收集自西元 2010 年一月至西元 2018 年二月於國立臺灣大學生物資源暨農學院附設動物醫院內，透過細胞學或病理學診斷為多中心型淋巴瘤之犬隻病歷資料進行分析，最終共計 62 隻犬隻符合收案條件而納入研究。根據使用之療程類型，42 隻犬隻被分配至 25 週療程組；22 隻犬隻被分配至 15 週療程組。兩組在病患特徵和疾病臨床特性（包括臨床分期、次分期、可能與較差預後有關的指標如診斷時出現血小板低下、T 細胞免疫分型、、高血鈣）分佈上並無顯著差異。反應率方面，25 週療程與 15 週療程之整體反應率分別為 97.6%和 100%。其中，25 週療程組及 15 週療程組中分別有 83.3%及 95.5%的犬隻反應達到完全消退。無論整體反應率或各類反應分佈，在兩組之間並無統計顯著差異。25

doi:10.6342/NTU201801637

週療程之中位疾病進展時間（Time to progression）為 242 天，而 15 週療程為 217 天，兩者之間無顯著差異（P=0.503）。中位存活時間（Overall survival time）方面，25 週和 15 週療程分別為 354 及 326 天，兩者同樣無顯著差異（P=0.999）。毒性評估部分，全部的副作用發生事件中有 67.8%源自腸胃道，又以厭食、嘔吐出現的頻率相當且均高於下痢。其餘事件屬於血液方面毒性，以嗜中性球低下為主。不論何種類型副作用，均有超過 50%以上事件之毒性強度被歸類為第一至二級。各類副作用之發生率在兩組之間無顯著差異。預後因子部分，體重高於中位數之犬隻、黃金獵犬、出現異常胸腔影像者，被發現能達到完全消退的比率較低。能在治療時達到完全消退的犬隻，在單變數和多變數分析中顯示均有顯著較長的疾病進展時間。經歷過嗜中性球低下的犬隻，僅在單變數分析中顯示有顯著較長的存活時間。

總體而言，15 週療程大致與改良之威斯康辛麥迪遜大學 25 週療程，兩者之療效和毒性相當。因此，療效不受影響且無副作用增加的情況下，使用較短且劑量密集的療程預期能夠為病患及飼主帶來更多臨床益處及便利性。

關鍵字：CHOP 療程、化學治療、犬多中心型淋巴瘤

doi:10.6342/NTU201801637

ABSTRACT

Canine multicentric lymphoma is the most common lymphoproliferative disease in dogs. Multi-agent chemotherapy has been as the mainstay of treatment due to high response rate, durable remission and relatively long survival time. Among them, the modified University of Wisconsin-Madison (UW-Madison) protocol without maintenance (i.e. the 25-week protocol), which included vincristine, cyclophosphamide, doxorubicin and prednisolone (known as CHOP), has been extensively used. Although most patients generally respond well to the treatment, progressive diseases developed and the patients inevitably succumb to the disease. To improve the treatment efficacy and shorten the lengthy and costly treatment, modification of protocol in a more dose-intense fashion has been proposed, such as co-administration of chemotherapeutic agents, decreased interval of drug administration or dose escalation. A 15-week CHOP protocol was therefore reported, and comparable efficacy and well-tolerated toxicity were showed initially [1]. The purpose of this study is to further compare two protocols most commonly used in National Taiwan University Veterinary Hospital Animal Cancer Treatment Center (NTUVHACTC), the 15-week CHOP protocol and the 25-week protocol, regarding their efficacy and possibility of adverse events. Potential prognostic impact was also evaluated.

Medical records of dogs diagnosed with multicentric lymphoma by either cytology or histopathology evaluation in NTUVHACTC from January 2010 to February 2018 were included for comparison. A total of sixty-four dogs met the inclusion criteria and were enrolled. According to the treatment protocol each patient received, forty-two dogs are assigned to 25-week group and twenty-two dogs were assigned to the 15-week group, respectively. There was no significant difference in distribution of patient

doi:10.6342/NTU201801637

demographics and clinical characteristics including stage, substage and parameters possibly associated with poor prognosis (e.g. thrombocytopenia, T-cell immunophenotype and hypercalcemia). Objective response rate of 25-week and 15-week protocol was 97.6% and 100% respectively. Among them, 83.3% of dogs in 25-week group and 95.5% of dogs in 15-week group attained complete remission. No statistically significance was noted for either objective response rate or distribution of each response between two groups. Median time to progression was 242 days in 25-week group and 217 days in 15-week group, which was showed no significantly different (P=0.503). Median overall survival time was 354 days in 25-week group and 326 days in 15-week group, without statistical significance presented (P=0.999). For adverse events, 67.8% of episodes were gastrointestinal in nature, with anorexia and vomiting equally and both more frequently presented than diarrhea. Rest of episodes were hematological in nature, majorly consisting of neutropenia. More than 50% of episodes were grade 1 to 2 toxicity, regardless of type of adverse events. No significant differences for the adverse event were noted between two groups. In the aspect of prognostic factors, dogs with body weight higher than median value, Golden retrievers and presence of abnormal thoracic image had significantly lower rate of complete remission. Dogs attaining complete remission had significantly longer time to progression in univariate and multivariate analysis. Dogs experiencing neutropenia were associated with significantly longer overall survival time only in univariate analysis.

In conclusion, the 15-week protocol was generally comparable to the modified UW-Madison 25-week protocol in both efficacy and adverse events. Therefore, a shorter, dose-intense protocol with similar efficacy and toxicity profile can bring more clinical benefits and convenience for both patients and their owners.

doi:10.6342/NTU201801637

Keywords: CHOP protocol, chemotherapy, canine multicentric lymphoma

doi:10.6342/NTU201801637

CONTENTS

doi:10.6342/NTU201801637

doi:10.6342/NTU201801637

doi:10.6342/NTU201801637

LIST OF FIGURES

doi:10.6342/NTU201801637

LIST OF TABLES

doi:10.6342/NTU201801637

doi:10.6342/NTU201801637

ABBREVIATION LIST

Abbreviations	Description	Abbreviations	Description
ATLS	Acute tumor lysis syndrome	ORR	Objective response rate
AE	Adverse event	OST	Overall survival time
BAL	Bronchoalveolar lavage	PARR	Polymerase chain reaction for antigen receptor rearrangement
BSA	Body surface area	PCR	Polymerase chain reaction
CR	Complete remission	PCV	Packed cell volume
CBC	Complete blood count	PD	Progressive disease
CHOP	Cyclophosphamide, Hydroxy-daunorubicin, Oncovin, Prednisolone	PD-1	Programmed cell death-1
CI	Confidence interval	PD-L1	Programmed cell death-ligand 1
CMOP	Cyclophosphamide, Mitoxantrone, Oncovin, Prednisolone	PR	Partial remission
DNA	Deoxyribonucleic acid	QBC	Quantitative blood count
FC	Flow cytometry	SD	Stable disease
G-CSF	Granulocyte colony-stimulating factor	SHC	Sterile hemorrhagic cystitis
GR	Golden retriever	TTP	Time to progression
IHC	Immunohistochemistry	VCOG	Veterinary Cooperative Oncology Group
ICC	Immunocytochemistry	WHO	World Health Organization
HR	Hazards ratio		
LR	Labrador retriever		
MCP-1	Monocyte chemotactic protein-1		
MRD	Minimal residual disease		
NTUVH	National Taiwan University Veterinary Hospital		

doi:10.6342/NTU201801637

Chapter 1 Literature review

1.1 Canine multicentric lymphoma

Lymphoma is one of the most common neoplastic diseases in pet dog population, with an annual incidence estimated to be 13-114 per 100, 000 dogs [2]. It comprises 7 to 24% of all canine neoplasia and 83% of all canine hematopoietic tumor [3], making it a significant disease in this species. Lymphoma consists of diverse groups of neoplasia, possessing heterogeneity regarding etiology, clinical and histopathological presentation, treatment response and prognosis. Among all the dogs diagnosed with lymphoproliferative diseases, 84% of the population develop multicentric lymphoma [3].

1.1.1 Signalment

For canine multicentric lymphoma, some species are well-known for increased risks for developing, including Bullmastiff, Rottweiler, Scottish terrier, Boxer, Cocker spaniel, German shepherd, Golden retriever (GR), Labrador retriever (LR) [2-5]. Even though any age dogs can be diagnosed with lymphoma, middle-aged or older dogs with the median age of 6 to 9 years are more likely to be affected [6]. Based on a large-scaled investigation for canine lymphoma in France where 608 cases were studied, the age at occurrence ranged from 1-16 years, with a mean of 8.3 years and a median of 8 years [5]. There is no such evidence of sex predisposition, although intact females associated with lower risk was once reported [6].

doi:10.6342/NTU201801637

1.1.2 Etiology

The etiology of multicentric lymphoma remains unclear and is thought to be multifactorial. Investigated factors include exposure to chemicals, waste incinerators, polluted sites and radioactive waste [3, 5]. DNA repair deficiency is also suspected, based on the finding of significantly increased chromosomal damage following ionizing radiation exposure and bleomycin exposure in lymphocytes of Golden retrievers diagnosed with lymphoma compared to which of normal Golden retrievers and mixed breed dogs [7].

1.1.3 Clinical presentation

Generalized, non-painful lymphadenopathy is the most common symptom in affected dogs. Clinically, most dogs show no signs. However, the swollen lymph nodes, especially those of mandibular or prescapular region, sometimes can still result in precaval syndrome, characterized by edema of head, neck and forelimbs, when the sizes become large enough to compress the circulation of local lymphovascular system (e.g. cranial vena cava). Dogs with precaval syndrome may suffer from dyspnea, noisy breathing sound, choking or difficulty of swallowing. Other nonspecific signs, such as anorexia, vomiting, depression, fever, or polydipsia and polyuria, may be seen as well and usually depend on the location and severity of organs involved.

Laboratory abnormalities associated with multicentric lymphoma included anemia, thrombocytopenia, neutrophilia, or circulating atypical lymphocytes which may indicate bone marrow involvement. Hypercalcemia may be noted as a paraneoplastic syndrome in approximately 10-40% of the clinical cases [8] and tends to associated with the production of parathyroid hormone (PTH)-related peptide and T-cell immunophenotype

doi:10.6342/NTU201801637

[8, 9].

Internal organ involvement can also result in abnormal imaging findings. In thoracic radiographs, abnormal pulmonary parenchymal patterns and enlargement intrathoracic (sternal, tracheobronchial and cranial mediastinal) lymph nodes are noted in 37% and 26 to 40% of dogs with multicentric lymphoma, respectively [10]. Pleural effusion may be presented as well. There are still other differentials accounting for the similar image changes, such as bronchopneumonia or chronic bronchial changes. However, so far, the information of the correlation or consistency between the abnormal imaging changes mentioned above and the cytological or histopathological findings from other diagnostic methods (i.e., bronchoalveolar lavage or lung biopsy) regarding determining pulmonary infiltration by multicentric lymphoma is scarce. Only one study in 1993 showed cytology finding of bronchoalveolar lavage (BAL) was more sensitive compared with radiographic evaluation of lungs, with 66% and 34% positive result, respectively [11]. In abdominal radiographs or ultrasonography, sublumbar lymphadenopathy, hepatomegaly and splenomegaly with echogenicity changes are three most common findings, ranging from 46 to 75% in dogs affected [10].

1.1.4 Staging

Evaluation of clinical staging is essential once the diagnosis of multicentric lymphoma confirmed on a dog. To provide the disease extent and tailor the treatment plan. The most widely adopted staging system is World Health Organization's clinical staging system for lymphoma in domestic animals proposed in 2011 by Valli *et al.* [3] (Table 1).

To define a clinical stage, information, such as hematology data, image details for liver and spleen, or bone marrow evaluation, is required. Among numerous diagnostic

doi:10.6342/NTU201801637

tools, application of more sensitive staging tests can detect previously unnoted lesions, especially those that are asymptomatic, and sometimes leads to stage migration. Substitution of abdominal radiography with ultrasonography and quantitative blood count (QBC) with complete blood count (CBC) both result in significant stage migration from less advanced to advanced stages (i.e. stage IV & V) [12]. Addition of splenic and hepatic cytology may help to distinguish between stage IV and III [13]; however, the addition of bone marrow cytology will not have a significant impact on staging [12]. Interestingly, even though the variable degree of stage migration is commonly noted among different studies, these changes of diagnostic methods exert no significant influence on either remission rate, remission duration, or survival in these studies [12, 13]. A possible explanation that disease extent may not be an essential determinant was proposed [13]. Therefore, application of additional diagnostic methods, especially more invasive ones, may be limited by the prognostic value in practice.

1.1.5 Immunophenotype

Immunophenotyping provides information of whether clonality exists and what type of cells comprising the disease. The ways of immunophenotyping include flow cytometry (FC), immunohistochemistry (IHC), immunocytochemistry (ICC) and polymerase chain reaction for antigen receptor rearrangement (PARR), according to the sample submitted [14-19]. For canine lymphoma, the distribution of B-cell, T-cell and unclassified subtype was estimated about 51.2 to 74%, 26 to 36% and 19.5%, respectively [18, 20, 21]. In the ability of correctly predicting immunophenotype (with IHC serving as a gold standard), FC was showed to be superior to PARR, with the sensitivity of the former significantly higher than the latter for both B-cell and T-cell. The percentage of agreement with IHC for FC and PARR was estimated to be 94% and

doi:10.6342/NTU201801637

70%, respectively [18]. However, if a fresh sample is not available or the result of flow cytometry is confounding, PARR is still highly suggested for immunophenotyping due to its high specificity of 98% [18].

1.2 Chemotherapy protocol

Chemotherapy is the mainstay for treatment of canine multicentric lymphoma. Significantly longer median survival time of 8 to 12 months can be achieved, compared to survival time of 1 to 7.5 months using steroid only and 4 to 6 weeks without treatment [3, 22]. Since lymphoma is chemosensitive, the goals of chemotherapy in these patients are aiming at a high response rate, a soon-achieved first complete remission and a durable response (> 6 months) [3].

Numerous chemotherapy protocols have been proposed and can be classified as single-agent or multi-agent therapy. Multi-agent protocol is the treatment of choice due to difficulty maintaining durable remission duration solely with one antitumor mechanism provided by a single agent (median survival time of 5.7 to 9 months for doxorubicin alone [23]). Multi-agent protocol is usually doxorubicin-based or refers to as CHOP protocol with drug combination of vincristine (Oncovin), cyclophosphamide, doxorubicin (Hydroxy-daunorubicin) and prednisolone.

For the past 20 years, modification of protocol while maintaining the same efficacy without further toxicity has been an important topic of multi-agent chemotherapy. Although high remission rate of more than 85%, traditional UM-Madison protocol with maintenance phase is thought to be lengthy and therefore money and time-consuming. Also, there is no inclusion of a maintenance phase in most human non-Hodgkin's lymphoma protocol [24]. Whether maintenance phase is necessary for protocol has been

doi:10.6342/NTU201801637

questioned. Based on several studies, a maintenance-free protocol can achieve similar response rate, remission duration and survival time without change of toxicity profile [25-29]. Therefore, a discontinuous protocol is more widely accepted now.

Because the outcomes of multicentric lymphoma patients did not improve much since the use of doxorubicin-based protocols starting in the1990s, and most patients still succumb to the disease and only very few long-term survivors encountered, another attempt of protocol modification focusing on dose intensity recently. To increase dose intensity, either increasing the frequency of administration by shortening the length of protocol and increasing the dose of specific chemotherapeutic agents meanwhile, or concurrent administration of two chemotherapeutic agents (i.e., vincristine and cyclophosphamide) in the same week were attempted [24, 28, 30]. The efficacy seemed to be similar to that of previous protocols and was not affected by the use of a shorter protocol, but the incidence of toxicity increased, and more treatment-related toxicities were noted [24, 28, 30]. Although these studies failed to demonstrate a better outcome as expected, administration of a short-term protocol without losing therapeutic benefit seemed to be possible.

To avoid the profound toxicity noted in other dose-intense settings, short-term, maintenance-free protocols without co-administration of chemotherapeutic agents, such as a 12 or 15-week protocol, were proposed [1, 24, 27]. The efficacy of these protocols is acceptable and comparable to that of other maintenance-free protocols. However, only one arm of the group was evaluated in those studies, and the comparison of response and survival data with historical reports concluded. It should result in certain limitations of reliability, such as bias due to an ununiform distribution of advanced stage patients between different studies. The outcomes, therefore, needed to be interpreted cautiously. The additional advantage of this short-term protocol is the relatively more

doi:10.6342/NTU201801637

extended treatment-free period during remission which represented a higher quality of life in patients and may increase owner compliance to some extent [27].

1.3 Prognostic factors

Many factors have been investigated for canine multicentric lymphoma to see if any prognostic value exists. Throughout most studies, factors can generally categorize into two types. The first type has identifiable findings from examinations at diagnosis, including T-cell immunophenotype, the presence of hypercalcemia, thrombocytopenia, anemia and bone marrow infiltration [31-37]. For bone marrow infiltration, both neoplastic lymphoid cells in peripheral blood and bone marrow are discovered by flow cytometry or PCR analysis. Although the degree of peripheral blood infiltration has directly correlated with that of bone marrow infiltration [35], it is the number of tumor cells in peripheral blood at diagnosis assessed by either quantitative PCR, real-time PCR or flow cytometry that is showed no significant correlation with prognosis [38, 39]. This phenomenon implies that the status of peripheral blood may not be able to sensitively and accurately reflect that of bone marrow every time or on every patient. However, the status of minimal residual disease (MRD), where the sequence of immunoglobulin from lymphoma cells was examined, in peripheral blood during the early phases of chemotherapy has directly associated with progression-free interval and overall survival [38]. This finding serves MRD in peripheral blood as a potential marker for early identifying patients who are more likely to relapse during the treatment and therefore aids in early initiation of a more dose-intense treatment before disease progression in these patients.

The second type is related to the clinical stage of a patient, with advanced stage

doi:10.6342/NTU201801637

and substage (i.e., stage V and substage b) being inferior factors [33, 35, 38, 40]. These factors both indicate possibly a more extent disease distribution in patients and subsequently poor clinical performance before treatment, limiting the use of an aggressive and dose-intense chemotherapy regimen considering patient's tolerability of toxicity.

The remaining factors more have something more to do with treatment, such as the history of steroid usage in pretreatment and response to treatment [33, 36, 40]. Use of steroid before chemotherapy leads to overexpression of drug efflux transporters (e.g., P-glycoprotein, multidrug resistance protein 1 and lung-resistance protein) on tumor cells and thus usually associates with drug resistance during treatment [41-43]. Other potential factors not routinely examined at the clinic but also showed prognostic value include overexpression of the chemokine monocyte chemotactic protein-1 (MCP-1) and hypermethylation of the *DAPK* CpG island [44, 45].

Recently, the role of checkpoint molecule expression (i.e., programmed cell death 1 and its ligand) has also been studied in canine lymphoma patient through examining the samples from fine needle aspiration by flow cytometry. It showed higher expression of programmed cell death 1 (PD-1) and programmed cell death-ligand 1 (PD-L1) in diseased dogs than in healthy animal [46]. Although the prognostic value of PD-L1 expression has not been established yet in canine lymphoma, the correlation between human diffuse large B-cell lymphoma and higher PD-L1 expression has been validated and carries a poor prognosis, with shorter overall survival in high expressers [47].

doi:10.6342/NTU201801637

Chapter 2 Introduction

Lymphoma is the most common hematopoietic tumor in dogs, comprising 7-24% of all canine neoplasia. Multicentric lymphoma serves 84% of dogs diagnosed with lymphoproliferative disease [3]. Chemotherapy has been the treatment of choice for its high response rate and most extended remission duration and survival time among all the treatment choices. Especially, multi-agent chemotherapy protocols have been showed to be more effective than single-agent use due to multiple different cytotoxic mechanisms in these protocols [3, 23, 48]. In traditional modified UW-Madison protocol without maintenance (i.e. 25-week CHOP protocol), the median remission duration was 220 days and median survival time was 303 days [25]. More dose-intense protocols such as 12-week or 15-week CHOP protocol were proposed to see if efficacy can remain with a shorter length of protocol, also fewer costs of time and money at the same time.

From one study where the 15-week CHOP protocol was first proposed [1], the response rate and overall survival time of the protocol were showed to be comparable with previous data, although shorter progression-free survival was noted. Application of this shorter, more dose-intense protocol was potentially feasible. However, comparison with other protocols through a second arm or control group has been lacking so far.

Since 15-week CHOP protocol has been used more in NTUVH for the recent years, the difference between the traditional 25-week protocol in actual efficacy and toxicity profile is also not extensively evaluated. Therefore, the purpose of this study was to compare the efficacy and toxicity of 15-week CHOP protocol and 25-week protocol in dogs diagnosed with canine multicentric lymphoma. Also, prognostic factors evaluated meanwhile. Comparable or even better outcome for 15-week protocol was expected.

doi:10.6342/NTU201801637

Chapter 3 Materials and methods

3.1 Patient selection

Dogs diagnosed with multicentric lymphoma and treated with a 15-week CHOP or 25-week CHOP/CMOP protocol were enrolled in this study. Medical records from January 2010 to February 2018 in National Taiwan University Veterinary Hospital (NTUVH) animal cancer treatment center were reviewed retrospectively. Patients eligible for inclusion in this study included dogs with a histological or cytological diagnosis of multicentric lymphoma, no previous therapy for tumor control except prednisolone and administration of either a 15-week or 25- week protocol as their initial chemotherapy. Criteria for cytology diagnosis included the predominance of medium to large lymphocytes constituting at least 50% of the total cell population. Use of L-asparaginase at induction of their CHOP protocol was acceptable. Dogs treated with any other chemotherapy agents or radiation therapy for lymphoma before the protocols begin were excluded from this study.

3.2 Data collection

The following information of cases, including breed, sex, weight, and age at diagnosis, stage, methods of staging, substage and status of steroid pretreatment, was collected. Immunophenotype and the results of complete blood count (CBC) and biochemistry panel at diagnosis were evaluated if available. Immunophenotyping was performed by either flow cytometry or immunohistochemistry.

doi:10.6342/NTU201801637

3.3 Stage

Cases were staged according to World Health Organization (WHO) clinical staging system for lymphoma in domestic animals [3] (Table 1).

Not every case had hepatic or splenic cytology and bone marrow biopsy evaluated routinely. For stage 4, either abdominal radiography or ultrasonography was used to evaluate liver/spleen involvement, requiring the finding of hepatomegaly and/or splenomegaly, with or without abnormal echogenicity in both organs. For stage 5, the following situations are suggestive of bone marrow involvement: 1. Presence of lymphocytosis in peripheral blood with the morphology of medium to large lymphocytes predominates in the blood smear. 2. Thrombocytopenia and anemia lack of presence of Ehrlichia canis and/or Babesia (i.e., *Babesia canis* and *Babesia gibsoni*) in blood smear, along with negative result in Ehrlichia antibody (IDEXX SNAP® 4Dx® Plus Test) or PCR test for Ehrlichia canis and Babesia (i.e., *Babesia canis* and *Babesia gibsoni*).

3.4 Chemotherapy

3.4.1 Protocol

A 15-week or 25-week chemotherapy protocol was administered as presented in Table 2. or Table 3., respectively. Dogs presented after 2016 were generally given a 15-week protocol after the design proposed by Thamm *et al.* was more well-studied [1, 24]. For 25-week protocol, there were two types of the setting of chemotherapy agents. One was the modified University of Wisconsin-Madison CHOP protocol without maintenance, and the other was similar to the general construction of the former but named as CMOP where substitution of mitoxantrone 6 mg/m^2 for doxorubicin was

doi:10.6342/NTU201801637

applied. The study reported by Wang *et al*. [49] showed CMOP provided similar efficacy to CHOP in both progression-free survival and median survival time. Therefore, dogs administered with either set of the 25-week protocol, or experienced drug switching from doxorubicin to mitoxantrone under the decision of the attending doctor during treatment were all included in this study.

Alteration of the protocol may be necessary during treatment under the following situations in NTUVH. Substitution of cyclophosphamide with chlorambucil at 20 mg/m^2 was performed when the patient was presented with sterile hemorrhagic cystitis suspected to the side effect of cyclophosphamide. In dogs with a high risk of developing acute tumor lysis syndrome (ATLS), dose reduction for the 1^{st} dose of vincristine in the first cycle of chemotherapy may be made from 0.7 mg/m^2 to 0.5 mg/m^2 according to the preference of the attending clinician. Due to the retrospective nature of this study, this dosage modification was acceptable and documented accordingly.

3.4.2 Monitor

At each visit for chemotherapy, a CBC was performed. Other parameters in biochemistry panel were performed based on the judgment from the attending clinician. A dose was delayed for seven days if the absolute neutrophil count was less than 3000/ μL, platelet count was less than 100,000/ μL, or any clinical condition where chemotherapy was contraindicated (i.e. grade 3-5 toxicity). Dose reduction ranging from 10 to 20% were performed at the discretion of the attending clinician according to the severity of previous adverse events.

doi:10.6342/NTU201801637

3.5 Response

Response to treatment was evaluated according to Response evaluation criteria for peripheral nodal lymphoma in dogs (v1.0) proposed by veterinary cooperative oncology group (VCOG) [50]. The lymph node size was measured by caliper and documented in diameters from three dimensions. Complete remission (CR) was defined as disappearance of all measurable disease; partial remission (PR) was characterized by at least a 30% but < 100% decrease in the mean sum longest diameter of target lesions. The progressive disease (PD) was characterized by at least a 20% increase in the mean sum longest diameter of target lesions or development of a new lesion; The stable disease (SD) was determined when neither sufficient decrease to qualify for PR nor sufficient increase to qualify for PD.

3.6 Toxicity

Toxicity was evaluated from the outcomes of hematology, biochemistry profiles and clinical performance at each visit and subsequently graded from 1 to 5 based on Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE) [51] (Table 4).

3.7 Statistical analysis

For all patients, objective response rate (ORR) was calculated by dividing the number of cases experiencing CR or PR with the total number of cases treated. Time to progression (TTP) was calculated from the date of treatment initiation to the date of PD. Overall survival time (OST) was calculated from the date of treatment initiation to the date of tumor-related death or euthanasia

doi:10.6342/NTU201801637

Demographic distribution between the group of 15-week protocol and the group of 25-week protocol was analyzed to see if a significant difference existed. Continuous data including age, body weight and days between diagnosis and treatment initiation was analyzed by Mann-Whitney U test. Categorical data, including breed, sex, stage, substage, immunophenotype and the status of steroid pretreatment, were compared by Pearson's chi-square test. However, if the expected value of a given cell in the comparison was less than five, Fisher's exact test was used instead.

Among parameters for evaluating clinical outcomes, ORR between two groups were compared by Pearson's chi square test; median TTP and OST were analyzed by Kaplan-Meier estimation and the distribution was displayed by Kaplan-Meier curve. Difference of TTP and OST between two groups was analyzed by log-rank test. For TTP, cases were censored at the time when they were lost to follow-up or still remained progression-free until the study closure. For OST, cases which were dead from unrelated causes, lost to follow-up or still alive at the time of study closure were censored. However, cases that were lost follow up were considered to have died from their disease if they were known to be out of remission at their last follow-up.

The influence of potential prognostic factors on achieving CR or not, TTP and OST of all patients were analyzed by Pearson's chi-square test and log-rank test, respectively. Factors analyzed included age, body weight, breed, sex, stage, substage, presence of thoracic involvement in imaging, pattern of thoracic involvement, immunophenotype, hematologic and biochemistry parameters (i.e. presence of anemia, thrombocytopenia, lymphocytosis, leukocytosis and hypercalcemia, baseline globulin and baseline neutrophils), status of steroid pretreatment, presence of adverse events (i.e. neutropenia, thrombocytopenia, anorexia, vomiting and diarrhea), requirements for dose delay and dose escalation, the use of L-asparaginase in protocol, need for first dose adjustment for

doi:10.6342/NTU201801637

avoiding ATLS and the use of mitoxantrone in protocol. Univariate factors with $P \leq 0.05$ were further analyzed by multivariate analysis using the Cox proportional hazards model.

In order to compare the incidence and severity of adverse events (AEs) between two protocols, Pearson's chi-square test was used to analyze the presence and distribution of adverse events including grading of neutropenia, thrombocytopenia, anorexia, vomiting and diarrhea occurred during treatment.

Mainly, for the group of 25-week protocol, we still wanted to see the influence of mitoxantrone administration on achieving CR, TTP and OST. Therefore, data from the subgroup of CMOP and CHOP was analyzed by Pearson's chi-square test and Kaplan-Meier estimation for achieving CR, and TTP and OST, respectively.

All analyses were performed by SPSS v. 20 software and were considered significant at $P \leq 0.05$.

doi:10.6342/NTU201801637

Chapter 4 Results

4.1 Demography

4.1.1 Patient characteristics

Sixty-four dogs met the inclusion criteria between January 2010 and February 2018 and was enrolled in the present study. 42 dogs received the 25-week protocol as their initial treatment; 22 dogs were treated with the 15-week protocol. There were 18 (28%) mixed breed dogs, 16 (25%) Golden retrievers, 6 (9%) Welsh corgis, 4 (6%) Schnauzers, 3 (5%) Beagles, 3 (5%) Malteses, 3 (5%) Shi-tzu, 2 (3%) West highland white terriers, 2 (3%) Poodles, and the rest included 1 Dachshund, 1 Labrador, 1 Husky, 1 Cavalier King Charles Spaniel, 1 Sheltie, 1 Shiba inu and 1 Yorkshire. The most common breeds in two groups both were mixed breed dogs and Golden Retrievers (Table 5). Median age was 7.8 (range 2-13 years) in the 25-week group and 9.5 (range 4-12 years) in 15-week group. Median body weight was 16.15 kg (range 5.06-54.4 kg) in 25-week group and 15.95 (range 1.46-40 kg) in the 15-week group. There were 17 spayed females, 5 intact females, 14 castrated males and 6 intact males in the 25-week group; 8 spayed females, 4 intact females, 9 castrated males and 1 intact male in the 15-week group. There was no significant difference in the distribution of patient characteristics between two groups. Detailed information was summarized in Table 5.

4.1.2 Clinical stage

Fifty-six dogs had sufficient information for staging, with 8 dogs in the 25-week group lack of information for determining liver or spleen involvement. At diagnosis, a blood work at least including a CBC was performed in all dogs. In the 25-week group,

doi:10.6342/NTU201801637

both abdominal radiography and ultrasonography were available for evaluation in 9 dogs; only abdominal radiography or ultrasonography were available for 7 dogs and 12 dogs, respectively. In the 15-week group, only 1 dog had information of both abdominal radiography and ultrasonography. The rest were staged via abdominal ultrasonography for determination of stage IV. No dogs in either group performed a splenic or hepatic cytology. The thoracic image for pulmonary infiltration and intrathoracic lymph node evaluation was available in 27 (64.3%) dogs of the 25-week group and 20 (90%) dogs of 15 weeks. In 25-week group, 10 dogs had abnormal change in thoracic radiography, including pulmonary infiltration in 7 dogs; enlargement of sternal and tracheobronchial lymph node both in 6 dogs and widen cranial mediastinum in 4 dogs. In the 15-week group, 9 dogs were identified with abnormal findings, including 2 dogs with pulmonary infiltration, 3 dogs with enlarged sternal lymph node, 4 dogs with enlarged tracheobronchial lymph node and 3 dogs with widening cranial mediastinum.

Overall, among the 34 dogs available for complete staging in 25-week group, there were 1 dog (2.9%) in stage I, 8 (23.5%) dogs in stage III, 16 (47.1%) dogs in stage IV, and 9 (26.5%) dogs in stage V. The remaining 8 dogs without sufficient information were in either stage III or IV. In 15-week group, 2 (9.1%) dogs were in stage III, 14 (63.6%) dogs were in stage IV and 6 (37.3%) dogs were in stage V. No significant difference in stage allocation between two groups (P=0.408) (Table 5).

For substage, in the 25-week group 23 (54.8%) dogs and 19 (45.2%) dogs were classified as substage a and b, respectively. In the 15-week group, 12 (54.5%) dogs were substage a and 10 (45.5%) dogs were substage b. Most common clinical signs presented in dogs with substage b were lethargy, anorexia and panting. Other less frequent signs included ecchymosis, vomiting, diarrhea and vision impairment. No significant difference in substage distribution between two groups (P=0.987) (Table 5).

doi:10.6342/NTU201801637

4.1.3 Immunophenotype

Examination for immunophenotype was performed in 51 dogs. All dogs but 2 dogs from the 25-week group who were defined by immunohistochemistry were analyzed by flow cytometry. In 32 dogs available for immunophenotype from 25-week group, 28 (87.5%) dogs were B-cell; 1 (3.1%) dog was T-cell; 3 (9.4%) dogs were null type. In 19 dogs with available information from the 15-week group, 14 dogs (73.7%) were classified as B-cell; 2 (10.5%) dogs were T-cell; 3 (15.8%) dogs were null type. No significant difference in the distribution of immunophenotype between two groups (P=0.406) (Table 5).

4.1.4 Laboratory findings

More than half dogs from either group had no specific findings in laboratory examinations. Hypercalcemia as a possible paraneoplastic syndrome was only noted in 1 dog from the 25-week group among the whole population. Abnormal hematologic laboratory findings possibly associated with poor prognosis (e.g. thrombocytopenia and anemia) were not overrepresented in either group, for there was no significance recognized in the distribution of these factors between two groups (Table 5). Detailed features for two groups were listed in Table 6.

4.2 Chemotherapy protocol

Twenty-seven (64.3%) dogs from the 25-week group and 18 (81.8%) dogs from 15-week group were treated with the protocol on the same day of diagnosis. 5 (11.9%) dogs in 25-week group and 5 (22.7%) dogs in the 15-week group were pretreated with the corticosteroid. There was no significant difference for the distribution of days

doi:10.6342/NTU201801637

between diagnosis and treatment and numbers of population pretreated with steroid before chemotherapy between two groups (Table 5).

Particularly, the 1st dose adjustment for prevention of ATLS was more significantly presented in the 15-week group (4.8% in 25-week group versus 40.9% in the 15-week group, *P*=0.000) (Table 9).

4.2.1 Treatment of the 25-week group

Forty-two dogs were treated by the 25-week protocol. In respect of the construct of 25-week protocol, CHOP was used in 32 (76.2%) dogs and CMOP protocol was used in 7 (16.6%) dogs. 2 (4.8%) dogs were given 3 cycles of CMOP followed by 1 cycle of CHOP; 1 (2.4%) dog was given 3 cycles of CHOP and then 1 cycle of CMOP.

Thirty (71.4%) dogs completed the 25-week protocol. Among the 12 dogs who didn't finish the treatment, 4 dogs failed to retain complete remission through the current protocol and 8 dogs relapsed during treatment. Available information regarding rescue protocol for the 39 dogs who eventually relapsed included continuous incorporation of L-asparaginase into CHOP-based protocol (n=23), CCNU (n=2) and Dactinomycin (n=1).

Seven (16.7%) dogs were given L-asparaginase in the protocol. All dogs, except for 1 dog who was given 3 doses, received only 1 dose of L-asparaginase (10,000 IU/m^2, intramuscularly or subcutaneously). All doses were administered during treatment based on the judgement of the attending clinician, with none at the initiation of treatment as part of chemotherapy induction. The number of patients administered with L-asparaginase was not significantly different between two groups (Table 5).

Apart from the dose reduction for prevention of ATLS at initiation of chemotherapy, 13 (31%) dogs experienced a total of 20 dose reductions during treatment. Of these 13

doi:10.6342/NTU201801637

dogs, 9 dogs required one dose reduction, 2 dogs experienced dose reduction for twice; 1 dog for 3 times and 1 dog for 4 times. 16 (80%) events were due to neutropenia. As for preventive dose reduction for ATLS, 2 (4.8%) dogs whose large tumor burden recognized as a risk factor of developing ATLS received dose reduction of vincristine (0.5 mg/m^2) at treatment initiation.

Twenty-nine (69%) dogs experienced a total of 62 dose delays during treatment. 8 dogs required one dose delay, 12 dogs required two dose delays, 7 dogs required 3 dose delays, 1 dog required 4 dose delays and 1 dog required 5 dose delay. 43 (69.4%) episodes were due to neutropenia. 7 (11.3%) times were due to gastrointestinal toxicity. 3 (4.8%) episodes were due to elevated liver indexes. Other miscellaneous causes were such as leukocytosis, foreign body digestion before visit or owner's request. Median and mean dose delay were both 1.5 times (range: 0-5).

4.2.2 Treatment of the 15-week group

Twenty-two dogs were treated with 15-week protocol. Most dogs received the construct of CHOP, except for 1 dogs who was initially given mitoxantrone for the first 2 cycles in consideration of cardiac toxicity by the attending clinician according to the finding of heart murmur but then switched to Adriamycin after evaluation by a cardiologist and it turned out no previous concern. 16 (72.7%) dogs completed the protocol. Of the 6 dogs failing to complete, 1 was unable to reach complete remission, 2 were due to development of progressive disease during treatment and 3 were due to owner's financial concern. Available information regarding rescue protocol for the 13 dogs who eventually developed progressive disease included continuous incorporation of L-asparaginase into CHOP-based protocol (n=7), CCNU (n=1), and both of the above two ways (L- asparaginase followed by CCNU) (n=1).

doi:10.6342/NTU201801637

Two dogs were given L-asparaginase during treatment. One dog was given 1 dose and the other dog was given 2 doses due to multiple wounds on skin over the body and was therefore given L-asparaginase due to contradiction to chemotherapy. All doses were administered during treatment, rather than the treatment initiation.

Nine (40.9%) dogs experienced dose reduction during treatment. 5 dogs required 1 dose reduction, 3 dogs required 2 dose reductions and 1 dog required 4 dose reductions. As in 25-week group, most dogs were due to neutropenia. Other causes included gastrointestinal side effect (n=1), elevated liver indexes (n=1), or both (n=1). For prevention of ATLS, 9 (40.9%) dogs received dose reduction for 1st vincristine (0.5-0.6 mg/m^2) at treatment initiation.

A total of 30 episodes of dose delay were presented in 15 (68.2%) dogs. 8 dogs experienced 1 dose delay; 3 dogs required 2 dose delays; 2 dogs required 3 dose delays; 1 dog needed 4 dose delays and 1 dog needed 6 dose delays. 19 (63.3%) episodes were due to neutropenia; 5 (16.7%) were due to elevated liver indexes; 4 (13.3%) were due to gastrointestinal toxicity. The median and mean time of dose delay were 1 and 1.36, respectively (range: 0-6)

4.3 Outcome

4.3.1 Response

For the whole population in this study, ORR was 98.4%, with CR in 56 (87.5%) dogs, PR in 7 (10.9%) dogs and SD in 1 (1.6%) dog. In the 25-week group where ORR was 97.6%, 35 (83.3%) dogs reached CR; 6 (14.3%) dogs experienced PR and 1 (2.4%) dog experienced SD as their best response. In the 15-week group with ORR of 100%, 21 (95.5%) dogs reached CR and only 1 (4.5%) dog attained PR. There was no

doi:10.6342/NTU201801637

significant difference in the ORR between two groups (*P*=0.466) (Table 7).

4.3.2 Time to progression and overall survival

For all dogs, the median TTP was 231 days. In the 25-week group, the median TTP was 242 days (range: 23-1193 days). 2 dogs were lost to follow-up on Day 546 and Day 653 and censored on the day of last follow-up. 2 dogs still remained progression-free and were censored on the day of study closure (Day 878 and Day 530). In the 15-week group, the median TTP was 217 days (range: 28-563 days). 1 dog was lost to follow up on Day 56 and 5 dogs were still in remission by the day of study closure. The median follow-up duration for these 5 dogs was 180 days (range: 99-250 days). No significance was noted in median TTP between two groups (*P*=0.503) (Figure 1).

The median OST for the whole population was 354 days. The median OST in 25-week group was 354 days. 6 dogs were lost to follow-up and censored on the last day of presentation, with median follow-up duration of 498 days (range: 266-653 days). 1 dog died of unrelated cause (cardiac disease) on Day 878, and 3 dogs were still alive and censored by the closure of study on Day 530, 594 and 1193. The median OST in 15-week group was 326 days. 1 dog was lost to follow-up and censored on the day of last presentation (Day 56), 1 dog died of unrelated cause (cardiac disease) on Day 563, and 9 dogs were still alive and censored at the completion of the study with median follow-up duration of 250 days (range: 99-775 days). There was no significance in the median OST between two groups (*P*=0.999) (Figure 2).

The median follow-up duration for all dogs in the 25-week group and the 15-week group was 313 days (range: 126-1190 days) and 275 days (range: 56-775 days), respectively. No significant difference was observed in the follow-up duration between two groups (*P*=0.061).

doi:10.6342/NTU201801637

The median TTP and median OST for two groups were summarized in Table 8.

4.4 Adverse events

A total of 283 episodes of adverse events were noted for all 64 dogs in this study, with 192 ones from the 25-week group and 91 ones from the 15-week group. Hematologic AEs comprised 32.2% of total episodes, while gastrointestinal AEs made up 67.8% of all. 5 dogs didn't experience any toxicity, with 3 from the 25-week group and 2 from the 15-week group. Instead, 9 dogs experienced at least one grade 4 toxicity, with 5 dogs from the 25-week group and 4 dogs from the 15-week group. The detailed distribution of frequency and severity of adverse events was summarized in Table 9.

Among the AEs of hematologic in nature, 4 (57.1%) dogs in the 25-week group and 10 (45.5%) dogs in the 15-week group experienced neutropenia during treatment. Grade 1 neutropenia was the most frequently presented in both groups, accounting for 51% of neutropenia episodes in the 25-week group and 40% of that in the 15-week group. Grade 4 neutropenia was noted in 4 dogs from both groups, being 9.5% and 18.2% in each population, and among them only 1 dog from the 15-week group experienced grade 4 neutropenia for twice. Febrile neutropenia with grade 1 fever (39.9 ℃) was noted in 1 dog from the 15-week group on the third day after administration of 1st vincristine. The blood examination on the same day revealed grade 2 anemia (PCV=24.9%), grade 1 neutropenia (neutrophil=1581/μL) with decreased segmentation and mild toxic reaction, and grade 2 thrombocytopenia (platelet=84000/μL, by manual calculation). This dog recovered after administration of oral antibiotics (i.e. Augmentin). There was neither significance in the occurrence of neutropenia, nor occurrence of grade 4 neutropenia between two groups. (P=0.869 and 0.320, respectively). For

doi:10.6342/NTU201801637

thrombocytopenia, only 5 (21.4%) dogs in the 25-week group and 5 (22.7%) dogs in the 15-week group experienced the toxicity. Grade 1 thrombocytopenia predominated for both groups. Toxicity other than grade 1 was only noted in a minority of 23.5% of episodes from the 25-week group being grade 2. No significance was noted in the occurrence of thrombocytopenia between two groups (P=0.905). Generally, the frequency and severity of neutropenia were more profound than those of thrombocytopenia in both groups.

For AEs of gastrointestinal in nature, more than half of dogs in both groups experienced anorexia and vomiting. Grade 1 and grade 2 toxicity were in majority, with grade 2 anorexia and grade 1 vomiting being the most frequently presented situations in both two groups. Grade 4 toxicity was only noted once in 1 dog of the 25-week group with anorexia. No significance was noted in the occurrence of anorexia, vomiting and diarrhea between two groups (P=0.299, 0.749 and 0.487, respectively).

Two dogs from the 25-week group and 1 dog from 15-week group developed sterile hemorrhagic cystitis after first, second and third administration of cyclophosphamide, respectively. Cyclophosphamide was subsequently substituted by chlorambucil in the following cycles for these dogs.

One dog from the 15-week group developed dilated cardiomyopathy speculated to be resulted from cumulative cardiotoxicity of doxorubicin. This was a 5-year old Corgi. Panting and exercise intolerance had been noted for 1 month after last dose of doxorubicin injection, with the cumulative dose of doxorubicin being 180 mg/m^2. Deterioration of respiratory signs and presence of pleural effusion and ascites were noted at second month after last dose. Echocardiography revealed dilated cardiomyopathy and systolic failure, with poor fractional shortening of 15%. Clinical signs and effusion alleviated rapidly after administration of Pimobenan and Digoxin.

doi:10.6342/NTU201801637

Three days after discharge, this dog eventually died at home (one week after diagnosis of cardiomyopathy). Re-evaluation of echocardiography was thus unavailable.

Overall 20 dose reductions were observed in 13 (31%) dogs of the 25-week group and 15 dose reductions in 9 (40.9%) dogs from the 15-week group. The most common cause for dose reduction was neutropenia, accounting for 16 (80%) of episodes from the 25-week group and 8 (53.3%) episodes from the 15-week group. Other miscellaneous causes included gastrointestinal toxicity (n=6), elevated liver enzyme (n=3). There was no significant difference in the rate of dogs requiring dose reduction between two groups (P=0.326).

A total 62 episodes of dose delay from 29 (69%) dogs in the 25-week group and 30 episodes in 15 (68.2%) dogs from the 15-week group were noted. Neutropenia still remained the major cause for dose delay, accounting for 43 (69.4%) times of delay in the 25-week group and 19 (63.3%) times of delay in the 15-week group. Gastrointestinal toxicity resulted in 7 (11.3%) times of delay from the 25-week group and 4 (13.3%) times of delay from the 15-week group. Elevated liver indexes were associated with 3 (4.8%) episodes and 5 (16.7%) episodes from the 25 and 15-week group respectively. Other miscellaneous causes were such as leukocytosis, foreign body digestion before visit or owner's request. There was no significant difference in the rate of dogs requiring dose delay between two groups (P=0.385).

Other than the dose reductions meant for ATLS prevention at treatment initiation, 11 (26.2%) dogs from the 25-week group and 6 (27.3%) dogs from the 15-week group required neither dose reduction nor delay during treatment.

doi:10.6342/NTU201801637

4.5 Prognostic factors

4.5.1 Prognostic factors analysis for CR

The following factors were analyzed to see if they played a role in attaining CR or not: age (< or ≥ median age), body weight (< or ≥ median body weight), breed (Golden retriever or other breeds), sex (spayed female, intact female, castrated male or intact male), stage (< 5 or 5), substage (a or b), immunophenotype (B-cell, T-cell or null), administration of steroid before chemotherapy, presence of thoracic involvement in imaging, characteristics of thoracic imaging (infiltration or swollen lymph node), presence of laboratory abnormalities present at diagnosis (including anemia, thrombocytopenia, lymphocytosis and leukocytosis), baseline globulin (< or ≥ median value of globulin), baseline neutrophil (< or ≥ median value of neutrophil), presence and severity of AEs during treatment (including neutropenia, thrombocytopenia, anorexia, vomiting and diarrhea), need for dose delay, number of dose delay (< or ≥ 2 delays), need for dose reduction, number of dose reductions (< or ≥ 2 reductions), the use of L-asparaginase in protocol, dose reduction for ATLS prevention at treatment initiation, and the use of mitoxantrone in protocol. Hypercalcemia is not evaluated because only one dog has hypercalcemia among the whole population.

In univariate analysis of the above factors, body weight, breed and thoracic involvement in imaging were showed result in statistically significance in attaining CR or not during treatment. All dogs with body weight lower than median value (i.e. 16.15 kg) achieved CR; however, only 75% of dogs with body weight higher than median value attained CR (P=0.002). For dogs who were not Golden retrievers, the rate of CR was 95.8%, compared to that of only 62.5% in Golden retrievers (P=0.00048). Dogs without thoracic involvement in imaging were 100% CR, whereas only CR in 76.9%

doi:10.6342/NTU201801637

dogs with an abnormality in thoracic imaging (*P*=0.018). Interestingly, no significance was noted between different characteristics of thoracic imaging (85.7% rate of CR for infiltration versus 73.7% for swelling of lymph nodes, *P*=0.417).

Relatively higher rate of CR was shown in dogs presented with neutropenia during the treatment (94.1% versus 80.0%) and dogs encountering more than 2 times of dose delay (96.4% versus 80.6%), but both were not significant enough (*P*=0.088 and 0.057, relatively). The result of analysis was summarized in Table 10-12.

No statistical significance was found between dogs ever receiving mitoxantrone during the treatment and those who did not (*P*=0.517). Particularly in 25-week group, either the use of CHOP or CMOP resulted no significant difference in achieving CR (*P*=0.780).

4.5.2 Prognostic factors analysis for TTP

Factors mentioned previously in univariate analysis for attaining CR or not, along with response to treatment, were analyzed for TTP in all patients as well. Body weight, breed and response to treatment were factors shown to have significant influence on TTP (Table 13-15). Dogs with body weight higher than median value had significantly shorter median TTP than those whose body weight lower than median value (231 days versus 209 days, *P*=0.010). Golden retrievers had median TTP of 126 days which was significantly shorter than that of 244 days in other breeds (*P*=0.024). In dogs able to attain CR during the treatment, median TTP was 244 days and significantly longer than 56 days from those failed to achieve CR (*P*=0.000). Dogs with substage a also had relatively longer median TTP than those with substage b, but significance was not reached (273 days versus 209 days, *P*=0.069, Figure 6). The Kaplan-Meier curves for the above factors with significance were shown in Figure 3 to 5.

doi:10.6342/NTU201801637

In multivariate analysis for factors with significance (P<0.05) in TTP, only response to treatment remained statistically significant. Hazards ratio for dogs without CR and those who achieving CR was 3.560 (95% confidence interval, 1.433-8.842, P=0.006). Both body weight and breed were not significant (P=0.176 and 0.999, respectively) in multivariate analysis. Results of multivariate analysis with hazards ratio (HR) and 95% confidence interval (95% CI) of factors for TTP were summarized in Table 16.

No significance was found between dogs ever receiving mitoxantrone during the treatment and those who didn't (242 days versus 231 days, P=0.209). In the 25-week group, the median TTP between subgroup of CHOP and CMOP was revealed no statistically significant (242 days versus 273 days, P=0.274).

4.5.3 Prognostic factors analysis for OST

Factors mentioned previously in univariate analysis for TTP were analyzed for OST in all patients (Table 17-19). Body weight, breed, presence of neutropenia during treatment and response to treatment were significantly associated with median OST. With similar findings as in TTP, dogs with body weight higher than median value and Golden retrievers both had significantly shorter median OST, with 307 days versus 555 days (P=0.002) for the former subgroup and 245 days versus 496 days for the latter subgroup (P=0.007). Response to treatment remained significant for median OST as well, with 392 days in dogs attaining CR and 189 days in dogs without CR. In the aspect of adverse events from treatment, dogs ever experiencing neutropenia had significantly higher median OST (514 days versus 309 days, P=0.029). The Kaplan-Meier curves for the above factors with significance were shown in Figure 7 to 10.

doi:10.6342/NTU201801637

However, no factors with significance ($P \leq 0.05$) from univariate analysis remained significant in multivariate analysis for median OST. Results of multivariate analysis with hazards ratio (HR) and 95% confidence interval (95% CI) of factors for OST were summarized in Table 20.

For dogs ever receiving mitoxantrone during the treatment and those who didn't, median OST was not statistically significant between two subgroups (313 days versus 379 days, P=0.136). In 25-week group, the median OST for subgroup of CHOP and CMOP was 313 days and 379 days, respectively (P=0.176), without significance found.

4.5.4 Prognostic factors analysis for large-sized population and non-GR population

According to the results above, we further recognized the dogs with body weight over 20 kg from the other relatively small-sized dogs. In this subgroup of large-sized population, the rate of complete remission, TTP and OST were compared between Golden retrievers and other large-sized non-GR dogs. Detailed distribution for body weight was listed in Table 21. Although the rate of CR, median TTP and median OST were all numerically lower in GR than in large-breed non-GR dogs, statistical significances were not observed for either endpoint between two groups (Table 22).

Again, we eliminated Golden retrievers and recognized the subgroup of non-GR population. In this subgroup, the comparisons were focused between dogs < 20 kg and dogs ≥ 20 kg. No significant difference was noted in median TTP and OST (P=0.171 and 0.100, respectively) between two groups. However, the rate of complete remission was significantly higher in dogs < 20 kg than dogs ≥ 20 kg (100% vs. 81.8%, P=0.049) (Table 22).

doi:10.6342/NTU201801637

Chapter 5 Discussion

5.1 Outcomes of different protocols

For canine multicentric lymphoma, chemotherapy remains a primary treatment which has been by far the most effective way to achieve the best response and prolong the survival time to the most in these patients. Reported response rate generally ranges from 89% to 100% [1, 27, 30, 49, 52, 53]. Different protocols had been proposed, and the efficacy of a protocol sometimes could be only assessed by comparison with that from historical or published data due to lack of a control group in the same study. To the author's knowledge, this should be the first study focusing on the comparison of the two widely used protocols for canine multicentric lymphoma, namely the modified 25-week UW-Madison protocol and the 15-week maintenance-free CHOP protocol, in the same study. In the present study, 42 dogs were given the 25-week protocol and 22 dogs were administered the 15-week protocol. The objective response rate of 15-week group (100%) was not significantly different from that of 25-week group (98.4%), and both were comparable to previous data of response rate in chemotherapy reported for canine multicentric lymphoma. This finding may more likely reflect and be related to the chemosensitive nature of lymphoma as long as chemotherapeutic agent, especially a multiagent protocol, is administered. In the research by Hosoya *et al*. [52], the substitution of doxorubicin with cytosine arabinoside in protocol did result in a significantly shorter median duration of first remission (94 days versus 174 days, $P < 0.01$), but such change actually didn't influence the initial response (92% versus 100%). Modification of protocol in a multi-agent chemotherapy setting for canine multicentric lymphoma may not dramatically change the rate of response attained as long as multiple different cytotoxic mechanisms are still provided.

doi:10.6342/NTU201801637

One of the primary intentions of this study is to see whether a more dose-intense and shorter protocol contributes to a comparable or even better outcome, through the setting of two-armed comparison. The 15-week protocol used in this study was first investigated in the study by Curran and Thamm [1]. In that study, the objective response rate was 98%, the median progression-free survival (PFS) was 176 days and the median OST was 311 days. A relatively short PFS was noted. Possible explanation provided by the authors was the fact that 50% of the patient population was substage b, hypercalcemia or stage V at the time of diagnosis, possibly indicating a population with poorer prognosis. Interestingly, there was also 50% of population in the present study met at least one of the criteria mentioned above possibly associated with a poor prognosis. However, the TTP and OST from the 15-week group (217 days and 326 days, respectively) here were longer than the data by Curran and Thamm above, and both were comparable to those from our 25-week group. Even so, it should be interpreted with caution when the comparison was made between studies which may be possibly highly biased due to different backgrounds and numbers of populations, difference in standard of diagnostic, staging and treatment procedures between institutions and the methods of response assessment. Overall, the outcome of a more dose-intense and shorter protocol was acceptable and comparable according to the data presented here.

The median TTP was 217 days for the 15-week group and 242 days for the 25-week group (P=0.503), without significance noted. However, among the censored individuals, dogs that hadn't reached progressive disease by the closure of this study were more frequently presented in the 15-week group with significance (4.8% in the 25-week group versus 22.7% in the 15-week group, P=0.029), although the distribution of all the censored data for TTP among two groups was not significantly different (P=0.08). This finding indicates that there is still possibility that the median TTP from

doi:10.6342/NTU201801637

the 15-week group potentially may be longer than the data currently available. Nevertheless, if that's the case, whether significance in TTP between two groups will exist is still not known. The same situation was noted in the analysis of median OST as well. For OST, all the censored data and those who were still alive and thus censored were both significantly more presented in the 15-week group (for the former 23.8% in the 25-week group versus 50% in the 15-week group, P=0.034; for the latter 7.9% in the 25-week group versus 40.9% in the 15-week group, P=0.002). Although the numbers of the median OST in both group (354 days for the 25-week group and 326 days for the 15-week group) were comparable to the previous published data, ranging from 10 to 12 months [3] and no significance was noted between two groups (P=0.999), the result may be different if longer follow-up duration for the same population in the 15-week group was allowed in the future.

Particularly in the 25-week group, the administration of CHOP or CMOP didn't result in significant differences in the response rate, median TTP (242 days versus 273 days) and OST (313 days versus 379 days). Similar findings were noted in the investigation by Wang *et al*. [49] as well, where CHOP or CMOP were given in each 22 dogs, resulting in median TTP of 222 days and 162 days (P=0.75) and median OST of 318 days and 242 days (P=0.63) , respectively.

5.2 Adverse events

In the present study, most of the adverse events were low-grade toxicities and generally well-tolerated, similar to previous findings. Although the incidence of adverse events among different studies were variable and couldn't be actually compared due to inconsistent ways of data presentation and calculation, there was still something in

doi:10.6342/NTU201801637

common. In terms of grading of toxicities, generally more than half of episodes fell between grade 1 to 2 in most studies [1, 24-26, 49, 52, 53], regardless of different multiagent chemotherapy protocols administered. Besides, only in protocols involving co-administration or escalated dose of chemotherapeutic agents were significantly greater treatment-related toxicity found. Higher frequencies of treatment delay and dose adjustment were accompanied as well [24, 28, 30]. In the study by Chun *et al*., 35% of population in high-dosed group experienced treatment delays, where dosage of cyclophosphamide and doxorubicin was increased to 250 mg/m^2 and 37.5 mg/m^2 respectively, while 43% required dose adjustments. Also, higher percentage of severe hematologic and gastrointestinal toxicity was noted, with incidence of 24% and 16% respectively [28]. In the study by Burton *et al*., 62.7% of adverse events occurred after co-administration of vincristine and cyclophosphamide, compared to 1.3 to 18.7% after other single agent only from the same protocol [24]. These findings demonstrated that both designs of protocol from the present study were not expected to result in intolerable toxicities during treatment due to no adoption of aggressive regimen strategies such as dose escalation and co-administration of chemotherapeutic agents.

Sterile hemorrhagic cystitis (SHC) was noted in 2 (4.8%) dogs from the 25-week group and 1 (4.5%) dog from the 15-week group after first, second and third administration of cyclophosphamide, respectively. Currently identified risk factors for developing sterile hemorrhagic cystitis include younger age at diagnosis, longer length of induction protocol and higher cumulative cyclophosphamide dose [54]. The prevalence of SHC was estimated to be 3.8% according to a large pharmacy database [54], where a total of 583 dogs with lymphoma prescribed with oral cyclophosphamide were identified. Furthermore, the prevalence was also found to be associated with the number of cyclophosphamide cycles according to the same database, with 2.8% for

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dogs receiving fewer than 10 cycles of cyclophosphamide treatments and 9.1% for dogs administered with 10 or more cycles. The incidence of SHC in the present study seemed to be higher based on the number of administration suggested above. Possible explanation included that the dosage of cyclophosphamide given in NTUVH generally started from 250 mg/m^2 which was mildly higher than the dosage (200 mg/m^2) from the protocol used in part of the dogs from that database, probably resulting a higher cumulative dose here even under the same total cycle of drug administration.

5.3 Prognostic factors

There have been several well-known prognostic factors in canine multicentric lymphoma. Stage V, substage b, T-cell immunophenotype, and presence of thrombocytopenia at diagnosis are factors commonly identified to be associated with both shorter TTP and OST [33-36, 38, 40, 55-57]. Presence of anemia at diagnosis and pretreatment with steroid are more related to shorter survival time [35, 56, 58].

Factors associated with achieving CR or not are less commonly investigated, probably due to the fact of generally high response rate to treatment as a nature of lymphoma. Only in a recent and large-scaled study by Childress *et al*. in 2018 were factors associated with response mainly analyzed. Presence of thrombocytopenia at diagnosis, higher baseline serum globulin concentration, and greater age at diagnosis were more likely to have partial remission in 98 dogs investigated [36]. In the present study, however, these factors were not identified. Instead, other than body weight and breed which would be discussed later, thoracic involvement in imaging at diagnosis was showed to related to a lower rate of CR (100% versus 76.9%, P=0.018), with dogs presented with abnormal imaging findings (either infiltration or swelling of lymph

doi:10.6342/NTU201801637

nodes) in thoracic less likely to achieve CR. Abnormalities in thoracic cavity may indicate a more extensive disease involvement or result in a poorer clinical performance at diagnosis. In some patient, respiratory clinical signs were also accompanied and led to the designation of substage b [59], a frequently reported factor associated with poorer prognosis. Due to the fact that the exact causes of the abnormal imaging findings was not routinely further confirmed in these dogs, there was possibility of causes other than lymphoma infiltration accounting for the same imaging finding. Also, the imaging information was only available in 47 dogs (21 dogs in the 25-week group and 26 dogs in the 15-week group), making the sample size smaller and the statistical power less when analyzing this subgroup. Therefore, interpretation of the result here should be taken cautiously.

In univariate analysis of prognostic factors associated with treatment response (CR or not), median TTP and median OST, dogs with body weight higher than median value and Golden retriever were both found to have significantly lower rate of CR and shorter TTP and OST. However, to the author's knowledge, both factors haven't been recognized as negative factors for canine multicentric lymphoma in any previous studies. On the other hand, although Golden retriever has been recognized as a breed well known for lymphoma development and a lifetime risk of nearly 1 in 8 was reported [7, 60], it hasn't been reported as a poor prognostic factor either. Besides, for canine multicentric lymphoma, no breed has been shown as a poorer responder yet. Interestingly, lack of significance for these two factors was showed by multivariate analysis for TTP and OST, which possibly meant that body weight and breed in the present study were two confounding factors with interaction between each other existed. It wasn't difficult to see the correlation that the population with higher body weight would be majorly comprised by large breed dogs such as Golden retrievers. So basically,

doi:10.6342/NTU201801637

there was probably a single population, mainly Golden retrievers, from the present study accounting for the presence of these two factors at the same time. Why this phenomenon was able to result in a statistically poorer outcome was speculated to be associated with two reasons. First, inaccuracy for body surface area (BSA) tended to be noted in individuals at extremes of weight. At this point, not only Golden retrievers and other large breed dogs were involved, but also obese patients. Although scarce information in veterinary medicine, the impact of extreme height and weight on BSA has been investigated in human medicine. It was found that dosing differences based on BSAs calculated by different formulas were most apparent when patients were at both higher height and weight [61, 62]. Moreover, for obese patients, it has been recommended to dose based on actual body weight instead of making ideal body weight-based empirical dose reduction, according to the finding that concerns about toxicity or overdosing based on the use of actual body weight was not found [63]. In the present study, some of the obese patients or large breed dogs were dosed by the BSAs with empirical reduction at initiation of treatment or during treatment when patients gaining weight (data not showed). Therefore, the efficacy of maximum-tolerated dose (MTD) chemotherapy may be interfered due to underestimation of BSA and subsequently under-dose in these patients. For the second reason, overrepresentation of Golden retriever in the present study was noted meanwhile. The percentage of Golden retriever was 23.8% in the 25-week group and 27.3% in the 15-week group, comprising 25.6% population in a whole. This percentage seemed to be mildly higher compared to the those of Golden retriever in other studies ranging from 7.3% to 22.7% [1, 24, 26, 28, 30, 49, 52, 53, 64-66], with only 3 studies over 20% [49, 64, 65]. Therefore, the less favorable clinical outcomes possibly coming from the effect of BSA underestimation may be magnified when the number of dogs affected overrepresented, resulting a

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statistically significance which hadn't been seen previously. When these confounding factors were controlled in multivariate analysis, only response to treatment remained significant in median TTP. On the other hand, we recognized the population with larger size (i.e. over 20 kg) from others, and further compared Golden retrievers and non-Golden retrievers within this subgroup, in the aspects of attaining CR or not, median TTP and median OST. Based on the findings of lack of significance in either analysis (Table 21), Golden retriever was still not considered as a poor responder to chemotherapy, even when compared with other dogs with similar size of body in this study.

Response to treatment (CR versus non-CR), as mentioned above as the only factor remaining significant in multivariate analysis for TTP, has been identified as a prognostic factor in other researches as well [1, 28, 30, 40]. In the study by Jagielski *et al*. in 2002, where 63 dogs were included, both significantly longer first remission duration and survival time (215 days versus 75 days and 266 days versus 129 days, respectively) were noted for dogs achieved CR [40]. In the initial research studying the 15-week protocol by Curran *et al*. [1], experiencing a CR as best response was also found to have a positive prognostic influence on both TTP (202 versus 41 days, $P<0.0001$) and OST (331 days versus 110 days, $P=0.0003$) in 134 dogs. In their study, this factor remained significant only for TTP rather than OST in multivariate analysis, a similar finding as our study. Based on this point, it was crucial to achieve CR as much as possible during the treatment. Even though multicentric lymphoma was a systemic disease, control of grossly measurable disease to the most extent still seemed to be beneficial for prolonging disease-free status and improving quality of life. As for OST, there were variable factors possibly influencing the outcome as well, such as the use of rescue protocol, the impact of delayed treatment-related toxicity from initial treatment

doi:10.6342/NTU201801637

(e.g. cumulative cardiotoxicity induced by doxorubicin) on clinical performance and owner's decisions, all making OST more easily biased than TTP in terms of efficacy evaluation of treatment. Accordingly, the effect of complete responder may not be able to show in OST.

The prognostic value of presence of treatment-associated adverse events had been identified in several studies, regardless of different chemotherapy protocols administered [1, 24, 30, 67]. Prolonged first remission duration was found in dogs either developing at least one grade III or IV neutropenia [67], or requiring hospitalization for adverse events [1]. Longer TTP and OST were noted in dogs requiring treatment delays or dose reductions during treatment than those who didn't [24, 30]. In the present study, the presence of neutropenia during treatment was associated with significantly prolonged OST (514 days versus 309 days, P=0.029) in univariate analysis. However, further analysis of the presence of either at least one grade III or IV neutropenia showed no statistical significance (P=0.069 and 0.253, respectively). Lack of significance in subgroups of more severe grading of toxicity in the present study may be related to the low number of dogs experiencing and episodes happened (17 dogs with 16 episodes for grade III neutropenia and 8 dogs with 9 episodes for grade IV neutropenia), contrary to the study by Vaughan [67] where 40 instances and 35 instances for grade III and IV neutropenia respectively were included in the analysis. The statistical power may be low here. For dogs experiencing adverse events, higher drug exposure was also expected, based on the assumption that there should be variation of drug metabolism and clearance between individuals [1]. For dogs without toxicity presented, the effective dose for tumor may be not reached and therefore less favorable outcomes in this population. Especially, the association between neutropenia and outcomes, rather than other kinds of toxicity, indicated that response of bone marrow to chemotherapy was

doi:10.6342/NTU201801637

able to mirror systemic exposure and serve as an indirect parameter for monitoring the responsiveness of tumor to dose given. Similar findings had been noted in several human studies in adjuvant chemotherapy of breast cancer [68-71]. Tailored dose modification according to toxicity may enable the achievement of maximum tolerated dose in individual. However, for dose escalation, standard of care had been inconclusive and not extensively explored in small animal. In human, there was a few studies focusing on the feasibility of neutrophil-guided dose escalation [72-75], with 10-25% escalation for patients without grade 3 and 4 neutropenia after standard-dosed treatment. The use of primary granulocyte colony-stimulating factor (G-CSF) support was sometimes involved, but increased risk of adverse events and myelodysplastic syndrome was also accompanied [72]. For small animal, currently no species-specific G-CSF product was available either. Therefore, for neutrophil-guided dose escalation without G-CSF support, the study by Drooger *et al*. and Edlund *et al*. showed both acceptable toxicity and feasibility. Notably, efficacy following these dose adjustments however was not mentioned. Fortunately, an ongoing trial (CHOP Dose Escalation for Canine Lymphoma) in Flint Animal Cancer Center of Colorado State University is recruiting canine multicentric lymphoma patients. They are going to assess the ability to successfully escalate drug doses in dogs treated with the 15-week CHOP chemotherapy using a standardized dose escalation protocol, and the effect on outcome and toxicity will be evaluated as well. Their results should be able to provide more information regarding the above issue in the future.

Other factors well-known to be prognostic, such as stage V, substage b, T-cell immunophenotype, thrombocytopenia and anemia, were not recognized in this study. For clinical stage, bone marrow biopsy was not performed in any dogs from this study, making the designation of stage V less confident. As previously reviewed, the status of

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peripheral blood may not be able to sensitively and specifically reflect that of bone marrow in the aspect of lymphoma infiltration according to the findings of flow cytometry and PCR [38, 39]. Therefore, dogs actually with bone marrow involvement but not showing abnormal findings in peripheral blood yet may be false-negatively staged as stage IV (or lower), making potentially less favorable outcomes mingled within those from dogs with true stage IV. Ultimately, the difference in outcomes between stage V and other stages turned less evident.

Substage in the present study, although was almost showed significant (P=0.069) in univariate analysis of TTP, was still not prognostic. For substage, there is no quantitative or objective criteria for determination. Inter-individual variation in substage determination due to subjective assessment of severity and different criteria adopted should be taken into consideration. In the study by Barber *et al*., a questionnaire was devised to ascertain the clinical parameters and attributes veterinary oncologists considered when assigning clinical substage designation to dogs with multicentric lymphoma [59]. Gastrointestinal, constitutional and respiratory signs were the most commonly identified clinical factors, with greater than 90% respondents indicating that inappetence, vomiting, diarrhea, changes in attitude, weakness and dyspnea were integral in assigning clinical substage. Metabolic, neurologic and nutritional parameters, however, were also considered in more than three-quarters of respondents. In terms of severity, mild-to-moderate severity of signs was reported to be sufficient for substage b designation. For studies in the future, by referring the parameters and criteria discussed above, synchronizing personal standard for substage designation can be expected, which should be helpful in minimizing the disagreement in substage from subjective evaluation as much as possible. The prognostic role of substage may be more likely to be established and concluded in that way.

doi:10.6342/NTU201801637

For immunophenotype, unavailability of information in a considerable number of dogs from both groups limited the statistical power. Also, breeds more predispositioned towards T-cell immunophenotype, such as Northern and Asian breeds (e.g. Shi-tzu, Husky and Welsh corgi) [4, 60] only presented in 11% population of this study, making it not surprising that the chance of identifying T-cell immunophenotype relatively low.

As for anemia at diagnosis, the prognostic value was mainly identified by two researches by Abbo *et al*., and Miller *et al*.[56, 58]. In the study by Abbo *el al*., the mean hematocrit of anemic dogs was 27.2% ± 6.47% (the median value was unavailable), which fell in the criteria of grade 2 anemia [58]. On the contrary, more than half of the anemic dogs were classified as grade 1 anemia in our study, consisting of 76% and 50% of anemic dogs from the 25-week group and the 15-week group respectively (Table 6). Also, in the research by Miller *et al*. where anemia was defined if PCV <40%, significance of anemia only showed in the group with PCV <35%, rather than the group with PCV 35-39%, a relatively mild severity for anemia according to their criteria [56]. These findings indicated that the impact of anemia at diagnosis on clinical outcome may not be obvious if only mild anemia presented at diagnosis. Similar distribution of severity was also noted for dogs with thrombocytopenia at diagnosis in the present study. 81.8% and 50% thrombocytopenic dogs from the 25-week and the 15-week group respectively were only grade 1 thrombocytopenia. Unfortunately, among the studies identifying thrombocytopenia as a prognostic factor, no further information about distribution of severity or mean/median value as mentioned above for anemia could be found for thrombocytopenia [24, 36, 65]. Most studies only mentioned the distribution of population categorized as being thrombocytopenia or normal. Therefore, it is still unknown whether the same assumption proposed for anemia that severity also counts for determination of the prognostic value could be applied to thrombocytopenia.

doi:10.6342/NTU201801637

5.4 Limitations

In this study, although two groups of population were from the same institute and shared the similar ways of medical care and management in tumor most of the time, there were still several limitations. Fundamentally, the retrospective and non-randomized nature prohibited the standardization of staging procedures, the criteria and management of dose adjustment and treatment delay, and evaluation of response and adverse events by one single observer continuously. Incompleteness and unavailability of information from medical records led to relatively low statistical power for the analysis in several subgroups. The decisions made by owner, which potentially depended on the willingness of aggressive treatment and financial concerns, also placed influence on acceptance of complete staging at diagnosis and overall outcome (e.g. pursue for rescue treatment). On the other hand, the case number of the 15-week protocol was still small due to relatively short foundation and adoption of this protocol in our institute. This limitation resulted in insufficient follow-up durations for nearly half (40.9%) of the population in the 15-week group who hadn't developed disease progression by the closure of study, causing data censored due to this reason more than expected.

doi:10.6342/NTU201801637

Chapter 6 Conclusion

For dogs treated with 15-week CHOP protocol in this study, comparable time to progression and overall survival time with 25-week modified UW-Madison protocol were showed. However, the result may be different if longer follow-up time is allowed, especially for dogs treated with 15-week protocol here, in the future. Adverse events were well-tolerated and the incidence and severity were similar between two groups. Experiencing toxicity such as neutropenia might imply a more effective drug exposure and resulted in a longer survival in the study. Dogs able to attain complete remission as best response during the treatment were expected to have longer remission duration and potentially overall survival time.

doi:10.6342/NTU201801637

Figures

doi:10.6342/NTU201801637

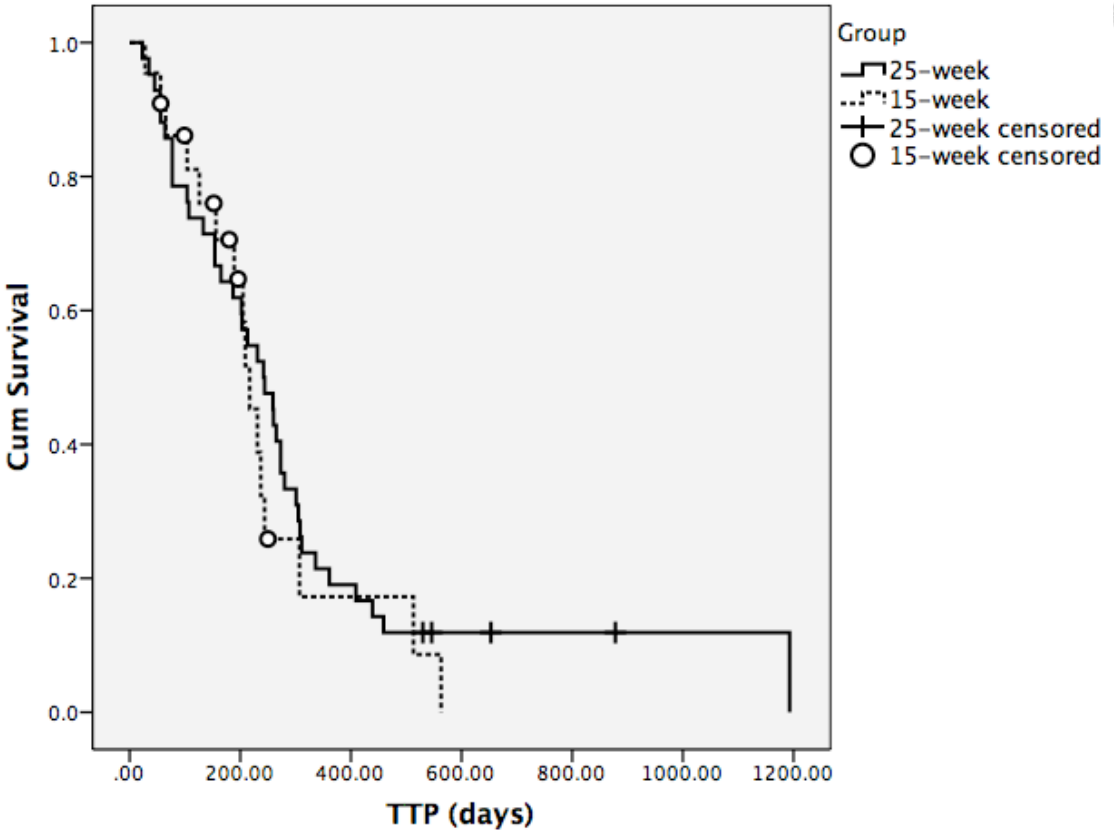

Fig. 1. The Kaplan-Meier curve for time to progression (TTP) of the two groups

The median TTP was 242 days for the 25-week group (solid line, n=42) and 217 days for the 15-week group (dashed line, n=22). No significant difference was observed (*P*=0.503).

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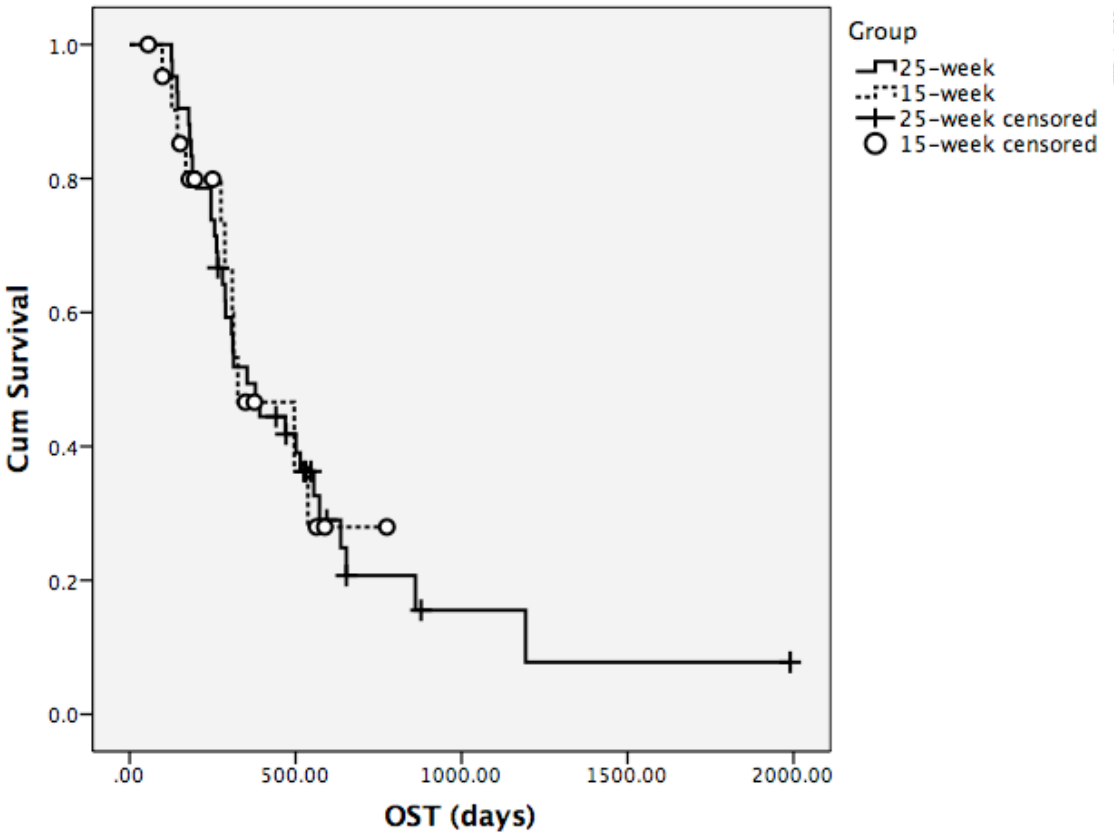

Fig. 2. The Kaplan-Meier curve for overall survival time (OST) of the two groups

The median OST was 354 days for the 25-week group (solid line, n=42) and 326 days for the 15-week group (dashed line, n=22). No significant difference was observed (*P*=0.999).

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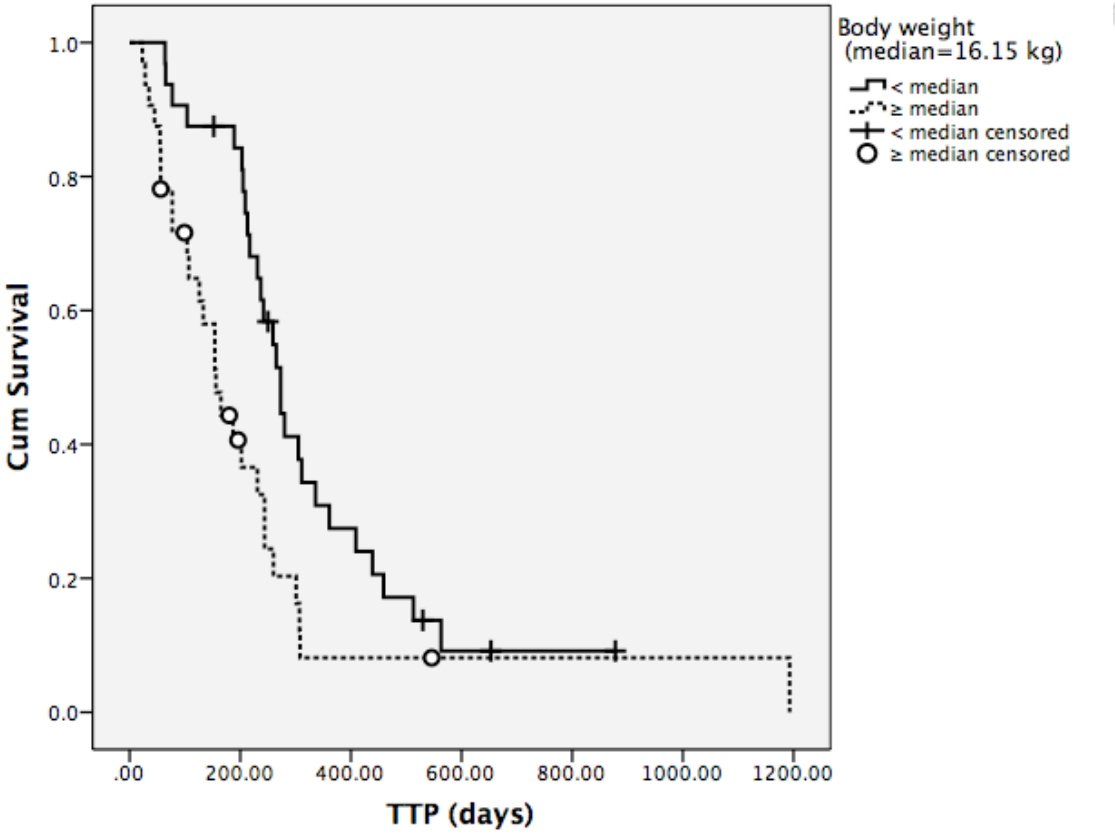

Fig. 3. The Kaplan-Meier curve of time to progression (TTP) for body weight (< and ≥ median body weight) from all patients

The median TTP was 273 days for dogs < 16.15 kg (solid line, n=32) and 156 days for dogs ≥ 16.15 kg (dashed line, n=32). Significant difference between two groups was observed (*P*=0.01).

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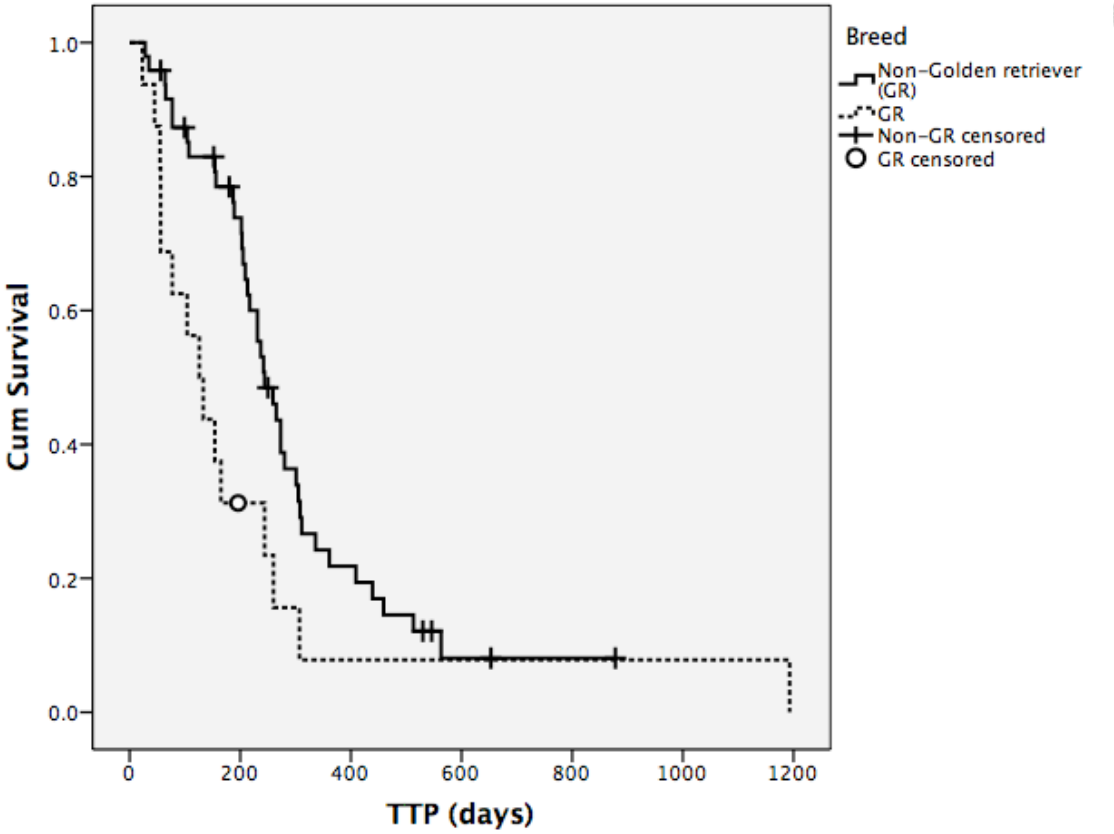

Fig. 4. The Kaplan-Meier curve of time to progression (TTP) for breed (Non-GR and GR) from all patients

The median TTP was 244 days for non-GR (solid line, n=48) and 126 days for GR (dashed line, n=16). Significant difference between two groups was observed (*P*=0.024).

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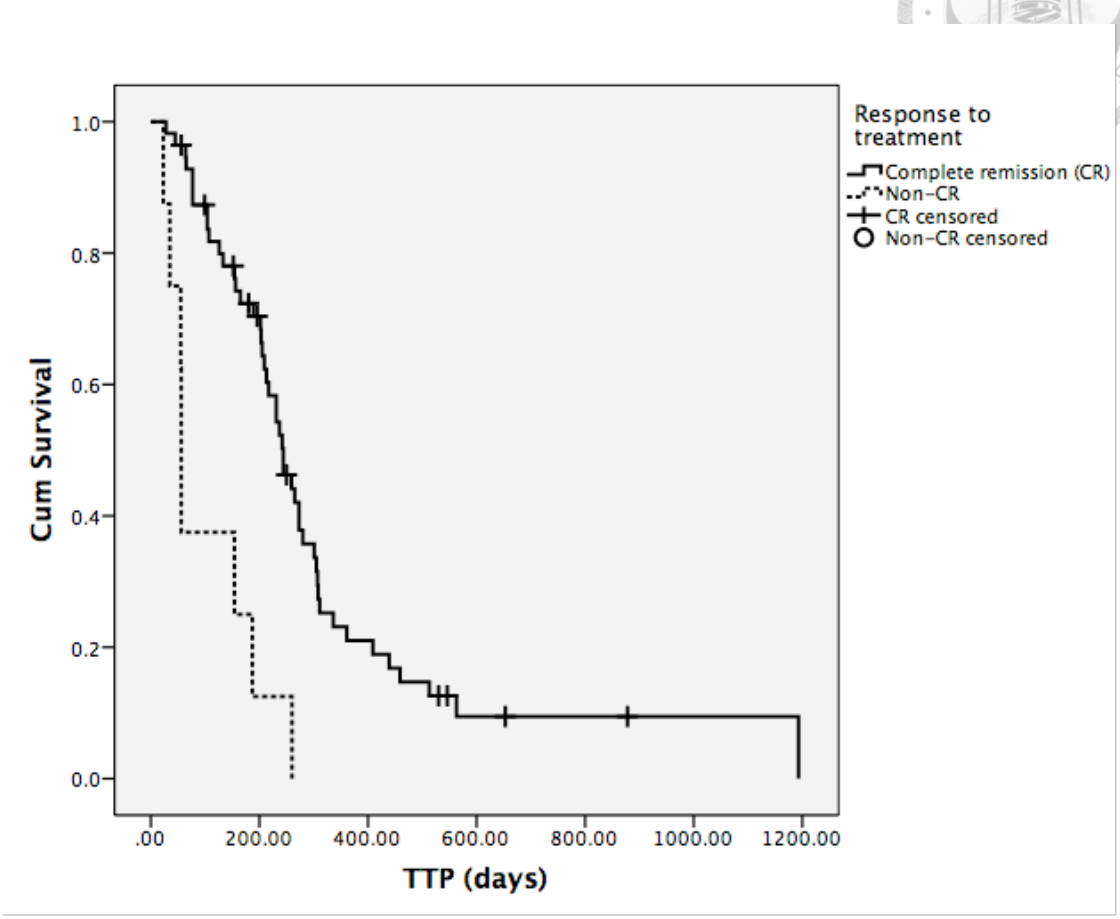

Fig. 5. The Kaplan-Meier curve of time to progression (TTP) for response to treatment (CR and Non-CR) from all patients

The median TTP was 244 days for CR (solid line, n=56) and 56 days for non-CR (dashed line, n=8). Significant difference between two groups was observed (*P*=0.000).

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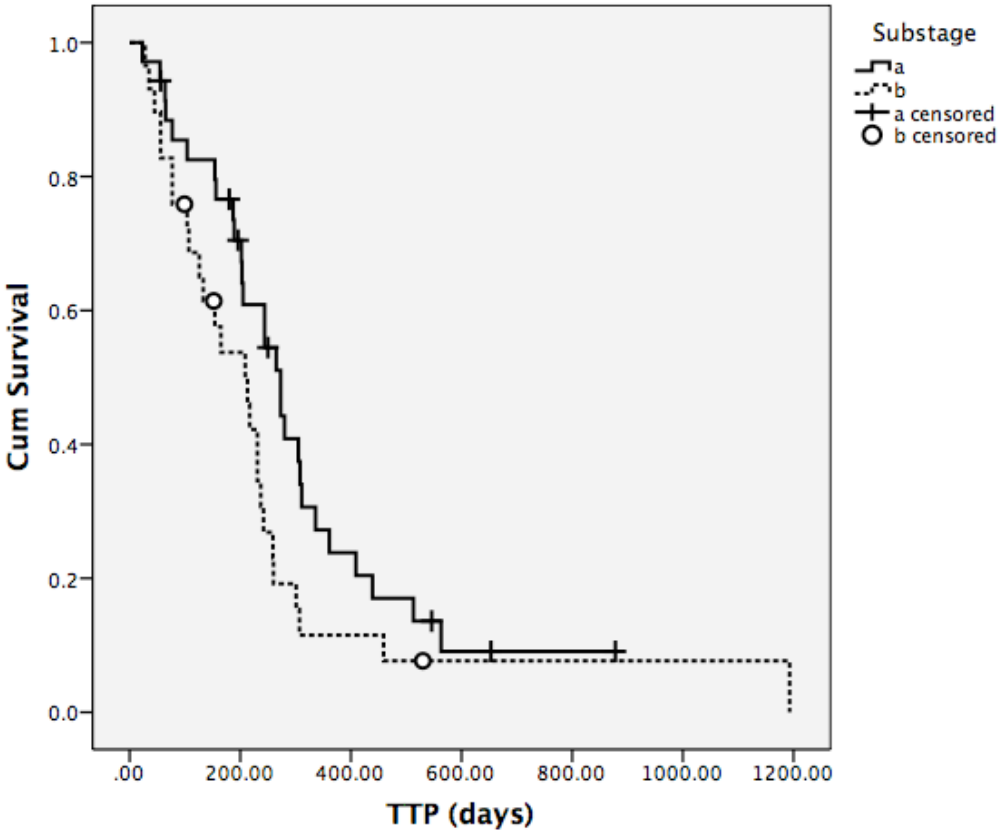

Fig. 6. The Kaplan-Meier curve of time to progression (TTP) for substage (a and b) from all patients

The median TTP was 273 days for substage a (solid line, n=35) and 209 days for substage b (dashed line, n=29). No significant difference between two groups was observed (*P*=0.069).

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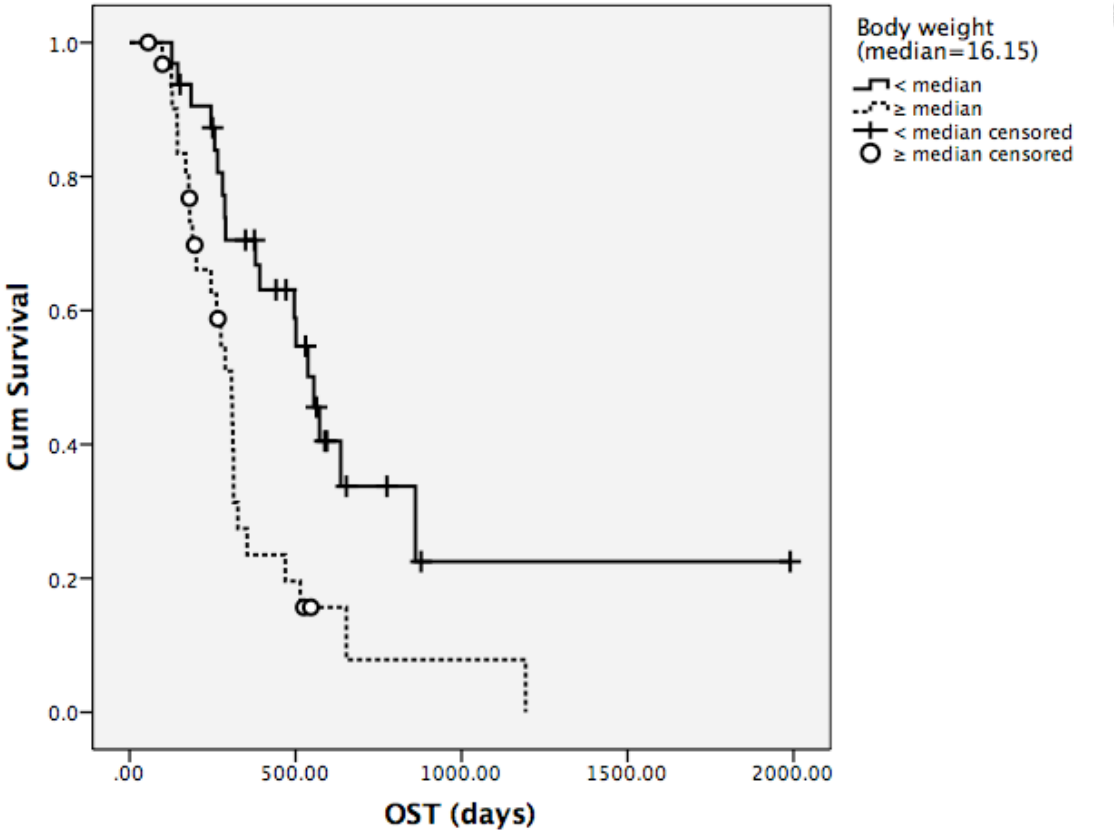

Fig. 7. The Kaplan-Meier curve of overall survival time (OST) for body weight (< and ≥ median body weight) from all patients

The median OST was 555 days for dogs < 16.15 kg (solid line, n=32) and 307 days for dogs ≥ 16.15 kg (dashed line, n=32). Significant difference between two groups was observed (*P*=0.002).

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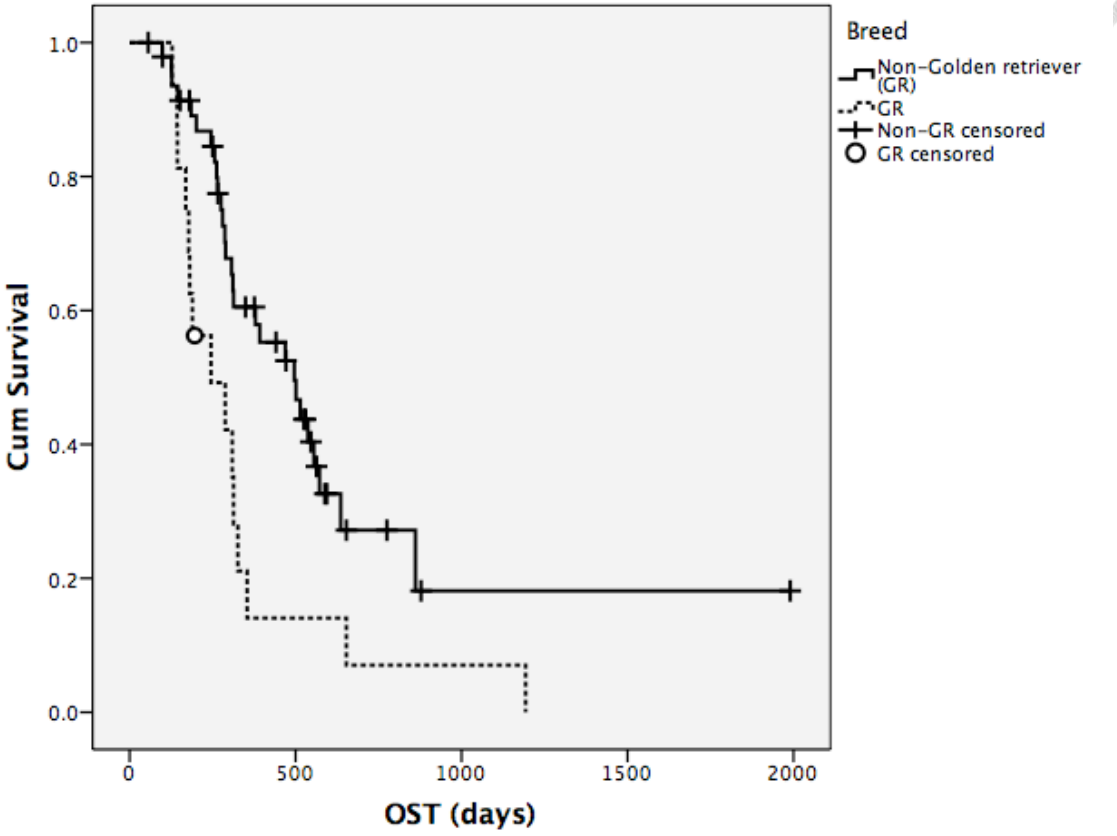

Fig. 8. The Kaplan-Meier curve of overall survival time (OST) for breed (Non-GR and GR) from all patients

The median TTP was 496 days for non-GR (solid line, n=48) and 245 days for GR (dashed line, n=16). Significant difference between two groups was observed (*P*=0.007).

doi:10.6342/NTU201801637

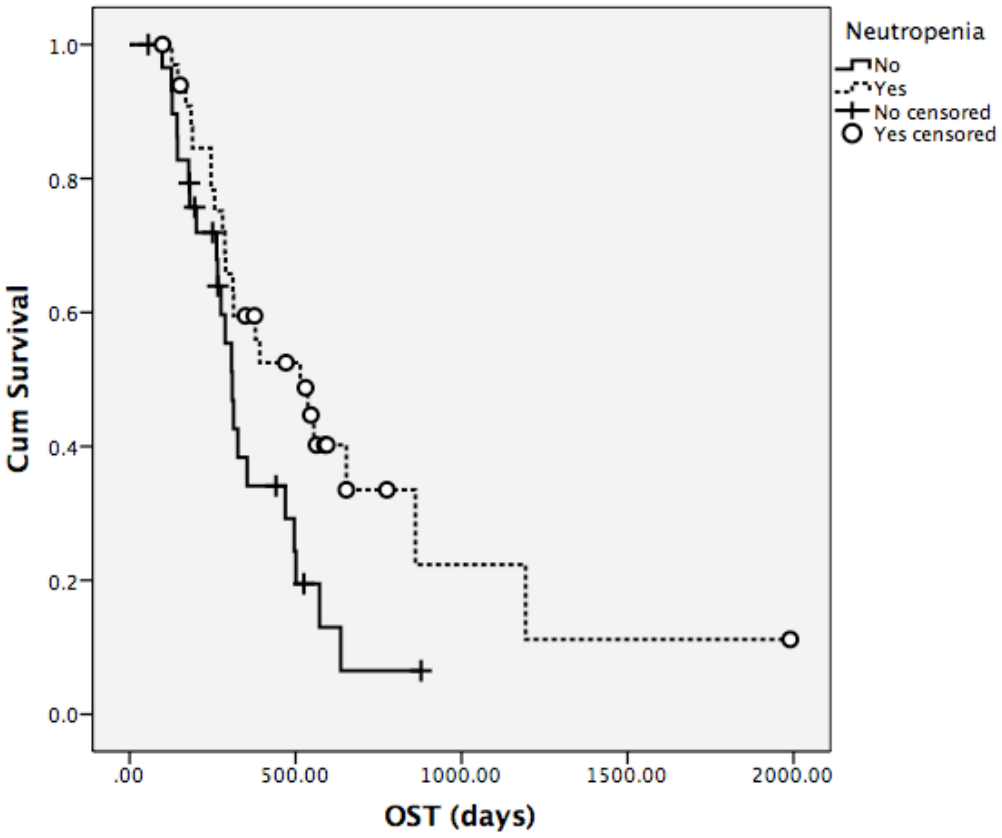

Fig. 9. The Kaplan-Meier curve of overall survival time (OST) for the presence of neutropenia during treatment from all patients

The median OST was 514 days for dogs experiencing neutropenia (absolute neutrophil count <3000 /uL) during treatment (solid line, n=34) and 313 days for dogs who hadn't experienced neutropenia (dashed line, n=30). Significant difference between two groups was observed (*P*=0.029).

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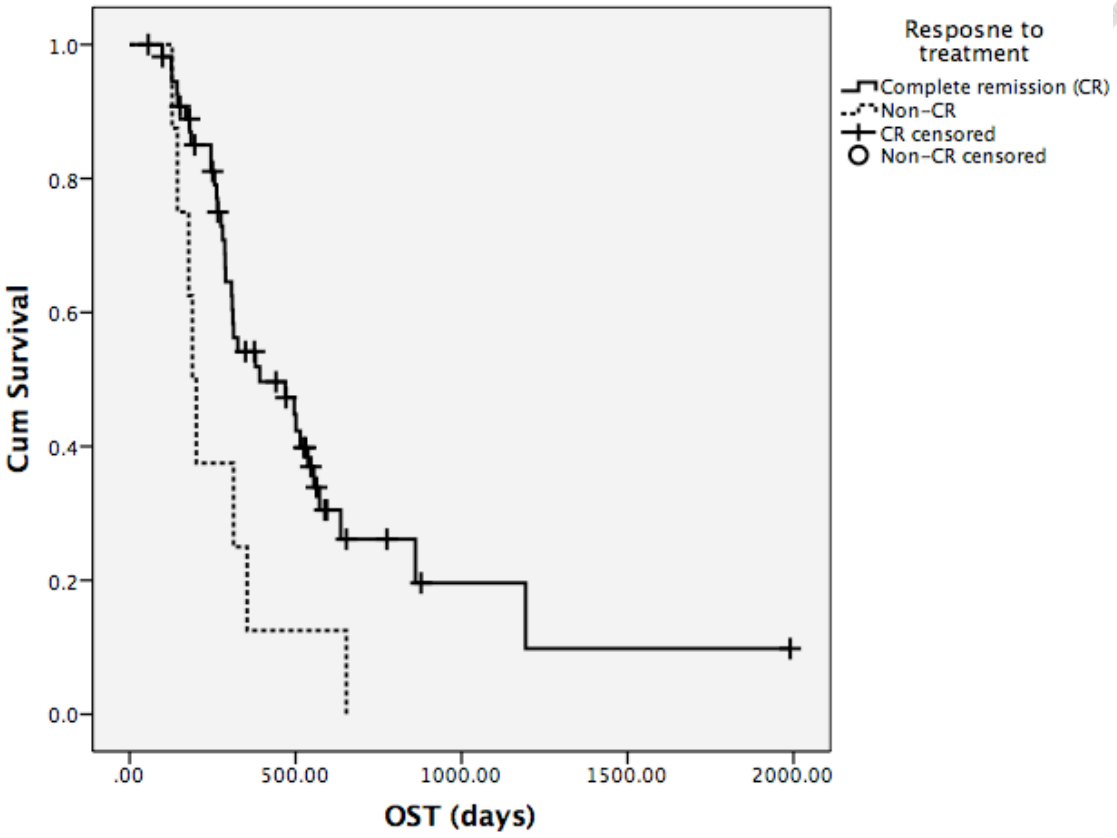

Fig. 10. The Kaplan-Meier curve of overall survival time (OST) for response to treatment (CR and Non-CR) from all patients

The median OST was 392 days for CR (solid line, n=56) and 189 days for non-CR (dashed line, n=8). Significant difference between two groups was observed (*P*=0.016).

doi:10.6342/NTU201801637

Tables

doi:10.6342/NTU201801637

Table 1. World Health Organization's clinical staging system for lymphoma in domestic animals

Stage	Criteria
I	Involvement limited to a single node or lymphoid tissue in a single organ [a]
II	Involvement of many lymph nodes in a regional area (± tonsils)
III	Generalized lymph node involvement
IV	Liver and/or spleen involvement (± stage III)
V	Manifestation in the blood and involvement of bone marrow and/or other organ systems, e.g. intestine, lung, neoplastic effusion (± stage I-IV)
Each stage is subclassified into:	
a	Without systemic signs
b	With systemic signs

[a] Excluding bone marrow.

doi:10.6342/NTU201801637

Table 2. 15-week CHOP protocol dosing schedule

Chemotherapy agents	Week												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**9**	**10**	**11**	**13**	**14**	**15**
Vincristine 0.7 mg/m^2 IV	◉				◉			◉			◉		
Cyclophosphamide 250 mg/m^2 PO		◉				◉			◉			◉	
Doxorubicin 30 mg/m^2 IV [a]			◉				◉			◉			◉
Prednisolone (mg/kg) Q24H PO	2.0	1.5	1.0	0.5									

[a] Patients with body weight <15 kg could receive doxorubicin at 1 mg/kg either

doi:10.6342/NTU201801637

Table 3. Modified University of Wisconsin-Madison protocol without maintenance (25-week protocol dosing schedule)

Chemotherapy agents	Week																
	1	2	3	4	5	6	7	8	9	11	13	15	17	19	21	23	25
Vincristine 0.7 mg/m^2 IV	⊙		⊙			⊙		⊙		⊙		⊙		⊙		⊙	
Cyclophosphamide 250 mg/m^2 PO		⊙					⊙				⊙				⊙		
Doxorubicin 30 mg/m^2 IV [a] or Mitoxantrone 6 mg/m^2 IV				⊙					⊙				⊙				⊙
Prednisolone (mg/kg) Q24H PO	2.0	1.5	1.0	0.5													

[a] Patients with body weight <15 kg could receive doxorubicin at 1 mg/kg either

doi:10.6342/NTU201801637

Table 4. Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (version 1.1)

Adverse events	Grade				
	1	2	3	4	5
Neutropenia	1500/ μL to <LLN	1000-1499/ μL	500-999/ μL	<500/ μL	Death
Thrombocytopenia	100000 /μL to <LLN	50000-99000/ μL	25000- 49000/ μL	<25000/ μL	Death
Anemia (Packed cell volume)	30% to <LLN	20 to <30%	15 to <20%	<15%	Death
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
Vomiting	<3 episodes 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. hemodynamic collapse)	Death
Diarrhea	Increase of up to 2 stools per day over baseline; no increase in frequency, however, consistency decreased over baseline	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. hemodynamic collapse)	Death

LLN, lower limit of normal; IV, intravenous; SC, subcutaneous; TP, total parental nutrition; PPN, partial parental nutrition; ADL, activities of daily living (eating, sleeping, defecating and urinating)

doi:10.6342/NTU201801637

Table 5. Comparison of characteristics between two groups

There was no significant difference in the distribution of patient and disease characteristics between two groups.

	25-week (n=42)	15-week (n=22)	*P* value
Age (years)			0.194
Median (range)	7.8 (2-13)	9.5 (4-12)	
Body weight (kg)			0.412
Median (range)	16.15 (5.06-54.4)	15.95 (1.46-40)	
Breed			0.434
Golden retriever	10 (23.8%)	6 (27.3%)	
Mongrel	14 (33.3%)	4 (18.2%)	
Other breeds	18 (42.9%)	12 (54.5%)	
Gender			0.583
Female spayed	17 (40.5%)	8 (36.4%)	
Female intact	5 (11.9%)	4 (18.2%)	
Male castrated	14 (33.3%)	9 (40.9%)	
Male intact	6 (14.3%)	1 (4.5%)	
Clinical Stage [a]			0.408
I	1 (2.9%)	0 (0%)	
III	8 (23.5%)	2 (9.1%)	
IV	16 (47.1%)	14 (63.6%)	
V	9 (26.5%)	6 (27.3%)	
Unavailable	8	0	
Substage			0.987
a	23 (54.8%)	12 (54.5%)	
b	19 (45.2%)	10 (45.5%)	
Hypercalcemia [b]			0.449
Yes	1 (2.6%)	0 (0%)	
No	38 (97.4%)	22 (100%)	
Immunophenotype [c]			0.406
B-cell	28 (87.5%)	14 (73.7%)	
T-cell	1 (3.1%)	2 (10.5%)	
Null	3 (9.4%)	3 (15.8%)	
Unavailable	10	3	
Use of L-asparaginase in protocol			0.707
Yes	7 (16.7%)	2 (9.1%)	
No	35 (83.3%)	20 (90.9%)	
Steroid pretreatment			0.257
Yes	5 (11.9%)	5 (22.7%)	
No	37 (88.1%)	17 (77.3%)	
Duration between diagnosis and treatment			0.144
Median (range)	0 (0-23)	0 (0-4)	
Thrombocytopenia at diagnosis [d]			0.469
Yes	11 (27.5%)	8 (36.4%)	
No	29 (72.5%)	14 (63.6%)	
Anemia at diagnosis			0.730
Yes	21 (50%)	12 (54.5%)	
No	21 (50%)	10 (45.5%)	
Baseline globulin [e] **(g/dL)**			0.508
Mean (range)	3.2 (2.2-4.7)	3.3 (2.4-3.8)	
Baseline neutrophil (/uL)			0.661
Mean (range)	9293 (3139-29211)	8976 (3542-28576)	

[a] 8 dogs in 25-week group were lack of information of abdominal image to determine liver/spleen involvement.

[b] Serum calcium concentration > 12 mg/dL. 3 dogs in 25-week group were lack of information of serum calcium concentration

[c] 10 dogs in 25-week group and 3 dogs in 15-week group were lack of information of immunophenotype.

[d] 2 dogs were unable to be assessed due to clotting sample.

[e] 1 dog in 25-week group and 2 dogs in 15-week group were lack of information of globulin at diagnosis.

doi:10.6342/NTU201801637

Table 6. Distribution of findings in laboratory examination at diagnosis between two groups

There was no significant difference in the distribution of abnormal laboratory findings between two groups.

	25-week (n=42)	15-week (n=22)	*P* value		25-week (n=42)	15-week (n=22)	*P* value
Anemia			0.730	**Lymphocytosis**	2 (4.8%)	3 (13.6%)	0.329
WNR[a]	21 (50%)	12 (54.5%)		**Leukocytosis**	9 (21.4%)	4 (18.2%)	1.000
Grade 1	16 (38.1%)	6 (27.3%)		**Hypercalcemia** [c]	1 (2.6%)	0 (0%)	0.449
Grade 2	4 (9.5%)	3 (13.6%)		**Neutrophil (/μL)**			0.661
Grade 3	1 (2.4%)	1 (4.5%)		Mean±SD	10881±5948	10388±6089	
Grade 4	0 (0%)	0 (0%)		Median (range)	9293 (3139-29211)	8976 (3542-28576)	
Thrombocytopenia [b]			0.469	**Globulin (/μL)** [d]			0.508
WNR[a]	29 (72.5%)	14 (63.6%)		Mean±SD	3.3±0.59	3.2±0.42	
Grade 1	9 (22.5%)	4 (18.2%)		Median (range)	3.2 (2.2-4.7)	3.3 (2.4-3.8)	
Grade 2	2 (5%)	4 (18.2%)					
Grade 3	0 (0%)	0 (0%)					
Grade 4	0 (0%)	0 (0%)					

[a] WNR: within normal range
[b] 2 dogs from 25-week group were unable to be graded due to clotting sample.
[c] Serum calcium concentration > 12 mg/dL. 3 dogs in 25-week group were lack of information of serum calcium concentration.
[d] 3 dog were lack of information of globulin at diagnosis

doi:10.6342/NTU201801637

Table 7. Summary of response to treatment in two groups

There was no significant difference in objective response rate and distribution of each response between two groups.

	25-week (n=42)	**15-week (n=22)**	***P* value**
Objective response rate [a]	41 (97.6%)	22 (100%)	0.466
Complete remission	35 (83.3%)	21 (95.5%)	0.364
Partial remission	6 (14.3%)	1 (4.5%)	
Stable disease	1 (2.4%)	0 (0%)	
Progressive disease	0 (0%)	0 (0%)	

[a] The percentage of complete and partial remission

doi:10.6342/NTU201801637

Table 8. Summary of median time to progression (TTP) and overall survival time (OST) for two groups

There was no significant difference in median TTP and OST between two groups.

	25-week (n=42)	**15-week (n=22)**	***P* value**
Median TTP	242 days	217 days	0.503
Median OST	354 days	326 days	0.999

doi:10.6342/NTU201801637

Table 9. Summary of adverse events and dose adjustment for two groups

There was no significant difference in the rate of occurrence for each adverse event between two groups. Most episodes from each event were grade 1 to 2. For dose adjustment, significantly more patients in 15-week group received dose reduction for ATLS prevention (*P*=0.000).

Adverse events	25-week (n=42)		15-week (n=22)		*P* value	Adverse events	25-week (n=42)		15-week (n=22)		*P* value
Neutropenia [a]						**Vomiting**					
Case experienced	24	(57.1%)	10	(45.5%)	0.869	Case experienced	25	(59.5%)	14	(63.6%)	0.749
Total episodes	49		20			Total episodes	72		29		
Grade 1	25	(51.0%)	8	(40%)		Grade 1	60	(83.3%)	23	(79.3%)	
Grade 2	7	(14.3%)	4	(20%)		Grade 2	11	(15.3%)	6	(20.7%)	
Grade 3	13	(26.5%)	3	(15%)		Grade 3	1	(1.4%)	0	(0%)	
Grade 4	4	(8.2%)	5	(25%)		Grade 4	0	(0%)	0	(0%)	
Thrombocytopenia [a]						**Diarrhea**					
Case experienced	9	(21.4%)	5	(22.7%)	0.905	Case experienced	13	(31%)	5	(22.7%)	0.487
Total episodes	17		5			Total episodes	19		9		
Grade 1	13	(76.5%)	5	(100%)		Grade 1	6	(31.6%)	6	(66.7%)	
Grade 2	4	(23.5%)	0	(0%)		Grade 2	10	(52.6%)	3	(33.3%)	
Grade 3	0	(0%)	0	(0%)		Grade 3	3	(15.8%)	0	(0%)	
Grade 4	0	(0%)	0	(0%)		Grade 4	0	(0%)	0	(0%)	
Anorexia						**Dose reduction**					
Case experienced	23	(54.8%)	15	(68.2%)	0.299	Case experienced	13	(31%)	9	(40.9%)	0.326
Total episodes	35		28			**Dose delay**					
Grade 1	11	(31.4%)	3	(10.7%)		Case experienced	29	(69%)	15	(68.2%)	0.385
Grade 2	20	(57.1%)	17	(60.7%)		**1st dose reduction for ATLS**					
Grade 3	3	(8.6%)	8	(28.6%)							
Grade 4	1	(2.9%)	0	(0%)		Case experienced	2	(4.8%)	9	(40.9%)	0.000*

[a] Neutropenia was defined as absolute neutrophil count <3000 /uL; thrombocytopenia was defined as platelet count <100,000 /uL.

* The exact *P* value was 0.00027

doi:10.6342/NTU201801637

Table 10. Univariate analysis of factors associated with demographics of patients in the whole population for attaining CR or not

The rate of complete remission was significantly lower in dogs with body weight higher than median value and Golden retrievers (P=0.002 and 0.000, respectively).

Factor	Number	% of complete remission	*P* value	Factor	Number	% of complete remission	*P* value
Age (years)			0.635	**Stage**			0.911
< 8 (median)	29	89.7		5	15	86.7	
≥ 8 (median)	35	85.7		< 5	49	87.8	
Body weight (kg)			0.002	**Substage**			0.296
< 16.15 (median)	32	100		a	35	91.4	
≥ 16.15 (median)	32	75		b	29	82.8	
Breed			0.000*	**Immunophenotype** [a]			0.628
Golden retriever	16	62.5		B	42	92.9	
Other breeds	48	95.8		T	3	100	
Sex			0.341	Null	6	83.3	
Female spayed	25	84		**Steroid pretreatment**			0.795
Female intact	9	88.9		No	54	87.0	
Male castrated	23	95.7		Yes	10	90.0	
Male intact	7	71.4					

[a] 13 dogs were lack of information of immunophenotype.

* The exact *P* value was 0.00048

doi:10.6342/NTU201801637

Table 11. Univariate analysis of factors associated with clinical findings in the whole population for attaining CR or not

The rate of complete remission was significantly different between dogs with thoracic involvement and those without (P=0.018). Dogs with thoracic involvement had significantly lower rate of complete remission.

Factor	Number	% of complete remission	*P* value	Factor	Number	% of complete remission	*P* value
Thoracic involvement [a]			0.018	**Lymphocytosis at diagnosis**			0.379
No	21	100		No	59	86.4	
Yes	26	76.9		Yes	5	100	
Pattern of thoracic involvement			0.518	**Leukocytosis at diagnosis**			0.725
Infiltration	7	85.7		No	51	88.2	
Swelling of lymph nodes	19	73.7		Yes	13	84.6	
Anemia at diagnosis			0.395	**Baseline globulin** [c]			0.478
No	33	90.9		< 3.3 (median)	30	90.0	
Yes	31	83.9		≥ 3.3 (median)	31	83.9	
Thrombocytopenia at diagnosis [b]			0.319	**Baseline neutrophil**			0.450
No	43	86.0		< 8976 (median)	32	90.6	
Yes	19	94.7		≥ 8976 (median)	32	84.4	

[a] 17 dogs were lack of information of thoracic radiography.
[b] 2 dogs were unable to be assessed due to clotting sample.
[c] 3 dog were lack of information of globulin at diagnosis.

doi:10.6342/NTU201801637

Table 12. Univariate analysis of factors associated with adverse events and treatment in the whole population for attaining CR or not

No factors associated with adverse events and treatment resulted in significant difference for attaining CR or not.

Factor	Number	% of complete remission	*P* value	Factor	Number	% of complete remission	*P* value
Neutropenia [a]				**Diarrhea**			
No	30	80.0	0.088	No	46	89.1	0.528
Yes	34	94.1		Yes	18	83.3	
< Grade 3	47	83.0	0.069	< Grade 2	52	88.5	0.628
≥ Grade 3	17	100		≥ Grade 2	12	83.3	
< Grade 4	56	85.7	0.253	**Dose delays**			
≥ Grade 4	8	87.5		No	20	80.0	0.221
Thrombocytopenia [a]			0.253	Yes	44	90.9	
No	50	90.0		< 2 delays	36	80.6	0.057
Yes	14	78.6		≥ 2 delays	28	96.4	
Anorexia				**Dose reduction**			
No	26	84.6	0.564	No	42	83.3	0.164
Yes	38	89.5		Yes	22	95.5	
< Grade 2	30	80.0	0.088	< 2 times	56	85.7	0.253
≥ Grade 2	34	94.1		≥ 2 times	8	100	
< Grade 3	54	87	0.795	**Dose reduction for ATLS**			
≥ Grade 3	10	90		No	53	86.8%	0.707
< 2 episodes	50	84.0	0.110	Yes	11	90.9%	
≥ 2 episodes	14	100		**Use of L-asparaginase**			
Vomiting				No	55	89.1%	0.341
No	25	88.0	0.923	Yes	9	77.8%	
Yes	39	87.2		**Use of mitoxantrone**			
< Grade 2	48	85.4	0.383	No	32	81.2	0.517
≥ Grade 2	16	93.8		Yes	10	90	
				25-week protocol [b]			
				CHOP	32	81.2%	0.780
				CMOP	7	85.7%	

[a] Neutropenia was defined as absolute neutrophil count <3000 /uL; thrombocytopenia was defined as platelet count <100,000 /uL.

[b] Factors only evaluated in 25-week group. Three dogs experiencing drug switch between doxorubicin and mitoxantrone were not purely CHOP or CMOP and thus not included.

doi:10.6342/NTU201801637

Table 13. Univariate analysis of factors associated with demographics of patients in the whole population for time to progression (TTP)

Time to progression was significantly shorter in dogs with body weight higher than median value and Golden retrievers (P=0.010 and 0.024, respectively).

Factor	Number	Median TTP (days)	*P* value	Factor	Number	Median TTP (days)	*P* value
Age (years)			0.872	**Stage**			0.591
< 8 (median)	29	231		5	15	231	
≥ 8 (median)	35	209		< 5	49	242	
Body weight (kg)			0.010	**Substage**			0.069
< 16.15 (median)	32	273		a	35	273	
≥ 16.15 (median)	32	156		b	29	209	
Breed			0.024	**Immunophenotype** [a]			0.675
Golden retriever	16	126		B	42	244	
Other breeds	48	244		T	3	202	
Sex			0.149[*]	Null	6	104	
Female spayed	25	237		**Steroid pretreatment**			0.347
Female intact	9	231		No	54	237	
Male castrated	23	259		Yes	10	202	
Male intact	7	165					

[a] 13 dogs were lack of information of immunophenotype.

[*] The *P* value between each two subgroups of the 4 gender statuses was only significant between the subgroup of intact male and castrated male (*P*=0.023)

doi:10.6342/NTU201801637

Table 14. Univariate analysis of factors associated with clinical findings in the whole population for time to progression (TTP)
No factors associated with clinical findings resulted in significant difference in median TTP.

Factor	Number	Median TTP (days)	*P* value	Factor	Number	Median TTP (days)	*P* value
Thoracic involvement [a]			0.613	**Lymphocytosis at diagnosis**			0.922
No	21	217		No	59	231	
Yes	26	209		Yes	5	231	
Pattern of thoracic involvement			0.333	**Leukocytosis at diagnosis**			0.564
Infiltration	7	336		No	51	237	
Swelling of lymph nodes	19	209		Yes	13	213	
Anemia at diagnosis			0.479	**Baseline globulin** [c]			0.272
No	33	237		< 3.3 (median)	30	217	
Yes	31	231		≥ 3.3 (median)	31	231	
Thrombocytopenia at diagnosis [b]			0.966	**Baseline neutrophil**			0.502
No	43	217		< 8976 (median)	32	231	
Yes	19	237		≥ 8976 (median)	32	217	

[a] 17 dogs were lack of information of thoracic radiography.
[b] 2 dogs were unable to be assessed due to clotting sample.
[c] 3 dog were lack of information of globulin at diagnosis.

doi:10.6342/NTU201801637

Table 15. Univariate analysis of factors associated with adverse events and treatment in the whole population for time to progression (TTP)

Dogs with complete remission had significantly longer TTP than dogs who failed to attain complete remission (*P*=0.000).

Factor	Number	Median TTP (days)	*P* value	Factor	Number	Median TTP (days)	*P* value
Neutropenia [a]				**Diarrhea**			
No	30	209	0.139	< Grade 2	52	217	0.472
Yes	34	242		≥ Grade 2	12	260	
< Grade 3	47	217	0.101	**Dose delays**			
≥ Grade 3	17	308		No	20	156	0.570
< Grade 4	56	217	0.088	Yes	44	244	
≥ Grade 4	8	459		< 2 delays	36	209	0.932
Thrombocytopenia [a]				≥ 2 delays	28	259	
No	50	237	0.309	**Dose reduction**			
Yes	14	165		No	42	244	0.458
Anorexia				Yes	22	203	
No	26	259	0.636	< 2 times	56	237	0.659
Yes	38	205		≥ 2 times	8	203	
< Grade 2	30	244	0.556	**Dose reduction for ATLS**			
≥ Grade 2	34	213		No	53	231	0.144
< Grade 3	54	237	0.802	Yes	11	217	
≥ Grade 3	10	213		**Use of L-asparaginase**			
< 2 episodes	50	237	0.918	No	55	237	0.193
≥ 2 episodes	14	202		Yes	9	154	
Vomiting				**Response to treatment**			
No	25	242	0.814	CR	56	244	0.000 *
Yes	39	217		Non-CR	8	56	
< Grade 2	48	237	0.890	**Use of mitoxantrone**			
≥ Grade 2	16	203		No	32	242	0.209
Diarrhea				Yes	10	231	
No	46	217	0.603	**25-week protocol** [b]			
Yes	18	260		CHOP	32	242	0.274
				CMOP	7	273	

[a] Neutropenia was defined as absolute neutrophil count <3000 /uL; thrombocytopenia was defined as platelet count <100,000 /uL.

[b] Factors only evaluated in 25-week group. Three dogs experiencing drug switch between doxorubicin and mitoxantrone were not purely CHOP or CMOP and thus not included.

* The exact *P* value was 0.000022.

doi:10.6342/NTU201801637

Table 16. Multivariate analysis of univariate factors with significance in the whole population for time to progression (TTP)

Dogs who failed to attain CR had significantly higher hazards ratio than those able to attain CR as their best response.

Factor	Number	Hazards ratio	95% CI	*P* value
Body weight (kg)		1.625	0.804-3.282	0.176
< Median	32			
≥ Median	32			
Breed		1.000	0.440-2.273	0.999
Other breeds	48			
Golden retriever	16			
Response to treatment		3.560	1.433-8.842	0.006
CR	56			
Non-CR	8			

doi:10.6342/NTU201801637

Table 17. Univariate analysis of factors associated with demographics of patients in the whole population for overall survival time (OST)

Overall survival time was significantly shorter in dogs with body weight higher than median value and Golden retrievers (P=0.002 and 0.007, respectively).

Factor	Number	Median OST (days)	*P* value	Factor	Number	Median OST (days)	*P* value
Age (years)			0.223	**Stage**			0.906
< 8 (median)	29	392		5	15	392	
≥ 8 (median)	35	309		< 5	49	326	
Body weight (kg)			0.002	**Substage**			0.237
< 16.15 (median)	32	555		a	35	501	
≥ 16.15 (median)	32	307		b	29	309	
Breed			0.007	**Immunophenotype** [a]			0.365
Golden retriever	16	245		B	42	392	
Other breeds	48	496		T	3	309	
Sex			0.529	Null	6	189	
Female spayed	25	469		**Steroid pretreatment**			0.231
Female intact	9	572		No	54	379	
Male castrated	23	392		Yes	10	311	
Male intact	7	289					

[a] 13 dogs were lack of information of immunophenotype.

doi:10.6342/NTU201801637

Table 18. Univariate analysis of factors associated with clinical findings in the whole population for overall survival time (OST)
No factors associated with clinical findings resulted in significant difference for median OST.

Factor	Number	Median OST (days)	*P* value	Factor	Number	Median OST (days)	*P* value
Thoracic involvement [a]			0.392	**Lymphocytosis at diagnosis**			0.612
No	21	392		No	59	313	
Yes	26	313		Yes	5	496	
Pattern of thoracic involvement			0.355	**Leukocytosis at diagnosis**			0.129
Infiltration	7	280		No	51	379	
Swelling of lymph nodes	19	313		Yes	13	289	
Anemia at diagnosis			0.517	**Baseline globulin** [c]			0.682
No	33	469		< 3.3 (median)	30	379	
Yes	31	307		≥ 3.3 (median)	31	326	
Thrombocytopenia at diagnosis [b]			0.952	**Baseline neutrophil**			0.538
No	43	379		< 8976 (median)	32	379	
Yes	19	354		≥ 8976 (median)	32	326	

[a] 17 dogs were lack of information of thoracic radiography.
[b] 2 dogs were unable to be assessed due to clotting sample.
[c] 3 dog were lack of information of globulin at diagnosis.

doi:10.6342/NTU201801637

Table 19. Univariate analysis of factors associated with adverse events and treatment in the whole population for overall survival time (OST)
No factors associated with adverse events and treatment resulted in significant difference for median OST.

Factor	Number	Median OST (days)	*P* value	Factor	Number	Median OST (days)	*P* value
Neutropenia [a]				**Dose delays**			
No	30	309	0.029	No	20	313	0.339
Yes	34	514		Yes	44	392	
< Grade 3	47	313	0.393	< 2 delays	36	313	0.586
≥ Grade 3	17	514		≥ 2 delays	28	501	
< Grade 4	56	313	0.188	**Dose reduction**			
≥ Grade 4	8	N/A[+]		No	42	354	0.961
Thrombocytopenia [a]				Yes	22	311	
No	50	354	0.508	< 2 times	56	379	0.724
Yes	14	309		≥ 2 times	8	280	
Anorexia				**Dose reduction for ATLS**			
No	26	496	0.179	No	53	379	0.516
Yes	38	311		Yes	11	313	
< Grade 2	30	496	0.205	**Use of L-asparaginase**			
≥ Grade 2	34	311		No	55	354	0.539
< 2 episodes	50	379	0.140	Yes	9	307	
≥ 2 episodes	14	289		**Response to treatment**			
Vomiting				CR	56	392	0.016
No	25	501	0.486	Non-CR	8	189	
Yes	39	313		**Use of mitoxantrone**			
< Grade 2	48	469	0.242	No	32	313	0.136
≥ Grade 2	16	289		Yes	10	379	
Diarrhea				**25-week protocol** [b]			
No	46	326	0.624	CHOP	32	313	0.176
Yes	18	354		CMOP	7	379	
< Grade 2	52	326	0.685				
≥ Grade 2	12	354					

[a] Neutropenia was defined as absolute neutrophil count <3000 /uL; thrombocytopenia was defined as platelet count <100,000 /uL.
[b] Factors only evaluated in 25-week group and three dogs experiencing drug switch between doxorubicin and mitoxantrone were not purely CHOP or CMOP and not included.
[+] 5 dogs were censored from the analysis, so the median OST was unavailable for this subgroup with only 3 dogs remained.

doi:10.6342/NTU201801637

Table 20. Multivariate analysis of univariate factors with significance for overall survival time (OST) in all patients

Although dogs experiencing neutropenia had lower hazards ratio than those who didn't, significance was not observed (*P*=0.092). No factors remained significant in multivariate analysis for OST.

Factor	Number	Hazards ratio	95% CI	*P* value
Body weight (kg)		1.804	0.784-4.150	0.165
< Median	32			
≥ Median	32			
Breed		1.366	0.579-3.225	0.476
Other breeds	48			
Golden retriever	16			
Neutropenia		0.573	0.300-1.096	0.092
No	30			
Yes	34			
Response to treatment		1.385	0.574-3.340	0.469
CR	56			
Non-CR	8			

doi:10.6342/NTU201801637

Table 21. Body weight distribution in large-sized dogs (≥ 20 kg)

Number	Golden retriever (kg)	Large-sized non-GR (kg)
1	24.2	20.6
2	26.9	20.6
3	27	23.3
4	27.8	24.5
5	28.3	24.9
6	28.3	25.3
7	29.2	29.4
8	30	30.6
9	31.3	33.4
10	33.9	44.6
11	34.2	54.4
12	34.8	
13	35	
14	40	
15	46	
16	47.4	

doi:10.6342/NTU201801637

Table 22. Comparison of rate of complete remission, time to progression (TTP) and overall survival time (OST) in three subgroups

Only the rate of complete remission was significantly lower in non-GR dogs whose body weight ≥ 20 kg than those whose body weight < 20kg (*P*=0.049). Other comparison didn't reveal statistical significance.

	Non-Golden retriever (non-GR)		**Golden retriever**
	Dogs < 20 kg	**Dogs ≥ 20 kg**	
Case number	37	11	16
Body weight (kg)			
Median (range)	10.6 (1.46-22.5)	25.3 (20.6-54.4)	30.65 (24.2-47.4)
P value	Not compared	0.126	
% of complete remission	100	81.8	62.5
P value	0.049	0.405	
Median TTP (days)	265	187	126
P value	0.171	0.413	
Median OST (days)	537	313	245
P value	0.100	0.370	

doi:10.6342/NTU201801637

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doi:10.6342/NTU201801637

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