Prognosis and risk factors in dogs with malignant lymphoma, a retrospective single-center study from 2005-2021 of 356 cases

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Abstract

Canine malignant lymphoma is a common neoplasm of the dog and although its frequent occurrence the cause is still not fully known. It generally causes severe disease, which in the majority of cases, eventually leads to death even when treated. Due to the severity of this disease, prognostication is crucial in order for the veterinarian to manage treatment and ensure quality of life for the patient.

The disease has proven itself to be a diagnostic and treatment challenge as the literature sometimes agrees, sometimes not, when discussing prognostic factors and demographic characteristics. Hence is why medical records from all dogs that visited UDS (University Animal Hospital) between 2005 and 2021 and diagnosed with malignant lymphoma (n=356) were examined for information on survival time, demographics, pathology and information on treatment. A multivariate statistical analysis (Kaplan-Meier and Cox Regression analysis) was used to determine the aim of the study: more specifically, what prognostic factors and demographic characteristics apply to those dogs with malignant lymphoma that visit UDS?

Kaplan-Meier estimates accompanied by Log-Rank and Wilcoxon-tests were deducted and found form of lymphoma (p=0.003, p=0.001), stage (p=0.001, p=0.000), substage (p=0.002, p=0.000), signs of paraneoplastic syndrome (p=0.036, p=0.002), dogs that received chemotherapy (p=0.000, p=0.000) and no treatment except for symptomatic treatment (p=0.000, p=0.000) to be significant for survival. This was followed by a Cox Regression that found mediastinal and multicentric form to have a RR for shorter survival of 2.7 and 2.3 respectively towards extranodal lymphoma. Stage V patients also showed a RR of 1.6 towards stage I-IV patients. However, it became evident that more research is needed as quite few uncensored values could be collected on mediastinal (n=10), alimentary (n=12) and extranodal lymphoma (n=14).

The conclusion and clinical application of the study was that the results mostly fit in with the general assumptions regarding canine malignant lymphoma but have in mind that it is a single-center study.

Keywords: Canine malignant lymphoma, malignant lymphoma, dog, prognosis, risk factors

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Abbreviations

FNA	Fine needle aspirate
MALT	Mucosa associated lymphoid tissue
MRD	Minimal residual disease
PARR	PCR for antigen receptor rearrangement
PFS	Progression-free survival
PTH	Parathyroid hormone
PTHrP	Parathyroid related protein
TK-1	Tyrosine kinase-1
UDS	University Animal Hospital

1. Introduction

Canine malignant lymphoma is one of the most common neoplasms in dogs, which according to Grüntzig et al. (2016) represents 4.35% of all the diagnoses of cancer, with an, according to Pinello et al. (2019), estimated incidence of 82/100,000 in male dogs and 70/100,000 in bitches.

Lymphoma originates from B- or T-lymphocytes, which are the cells that make up the body's adaptive immune system, and it manifests in different forms. It can be divided into four distinctive forms depending on in which organ it resides. Malignant lymphoma generally presents with elusive clinical signs depending on what organ systems have been affected and general lymphadenopathy is highly suggestive of the diagnosis. Santiago et al. (2022) claims lymphoproliferative neoplasia to be the most common cause of lymphadenopathy in the Mediterranean area, where the prevalence of infectious diseases, which also causes lymphadenopathy are especially high.

A diagnosis is made by a cytological evaluation followed by a thorough diagnostic work up including imaging, hematology and blood biochemistry. After a definitive diagnosis, cytostatic treatment is the option for most dogs, which puts most into remission for about 8-16 months.

The etiology of lymphoma is still unknown but is believed to be multifactorial. Some possible etiologies identified that may contribute to developing lymphoma include: a genetic component, exposure to chemicals, magnetic fields and/or ionizing radiation. (Zandvliet 2016)

Many risk factors are being discussed still, though it appears as if, according to Bennett et al. (2018), breed, sex, exposure to chemicals, magnetic fields and radiation and the fact that the dog is gonadectomized independently of sex has an impact. Equally important are the prognostic factors as these combined with the risk factors are what helps the clinician maneuver the patient through treatment, remission and relapse. The prognostic factors for the disease include: form of lymphoma affecting the dog, immunophenotype, grading and staging to specific symptoms of the paraneoplastic phenomena. (Zandvliet 2016)

Saellström et al. (2022) found that expression of the tyrosine-kinase-1 (TK-1) biomarker possibly could have some prognostic value in contrast to Falk (2018) who did not report this. Kiupel et al. (1999) have given an example of specific proliferative markers called AgNORs, that may contribute to prognostication.

The prognostic factors are going to be the main objective of this report, both possible positive and negative factors for survival time. Hopefully, the results will show what prognostic and risk factors apply to the demographic of dogs that come to University Animal Hospital (UDS) and the discussion that follows if they align

with the antecedent literature or not. In short, the aim of this study is to analyze what prognostic and demographic risk factors apply to dogs with malignant lymphoma, that visit UDS.

2. Literature review

2.1 Immune system

2.1.1 Anatomy of lymphoid organs

The bone marrow is located inside the cavity of the body’s long bones in the dog's extremities, foremost in the femur and humerus. At a young age, the color of the marrow is red, it has a gelatinous consistency and produces red and white blood cells. With age, this capacity subsides and the color shifts from red to a brownish yellow as fat is deposited within the marrow.

The thymus gland is an organ that normally regresses and becomes difficult to locate as the dog progresses through puberty. It has a lobulated structure and resides in the ventral cranial part of the mediastinum. It consists of a medulla and a cortex in which the T-lymphocytes are produced. The bone marrow and the thymus gland are considered the body’s primary lymphoid organs.

Lymph nodes are located throughout the body, some as shallow so they may be palpated, some buried under layers of tissue and some within the thorax and abdominal cavities. Lymphoma is generally detected by the owner as a noticeable swelling suddenly occurs in these. Throughout the intestinal tract, a diffuse or sometimes multifocal lymphoid tissue can be found within the wall of the tract known as mucosa associated lymphoid tissue (MALT). The mucosa of the small intestine holds focal patches of distinct lymphoid tissue known as Peyer’s patches.

The spleen is found cranially to the left in the abdomen where it follows the curvature of the abdomen ventrally to the right side. The tissue of the spleen is divided into red pulp which consists of blood vessels and cavities, and white pulp which consists of lymphoid tissue.

The lymph nodes, MALT and the spleen make up the body's secondary lymphoid organs.

2.1.2 Physiology of lymphocytes

Lymphoma originates from B- and T-lymphocytes located in solid organs such as lymph nodes, spleen, liver, skin, and eye. The B- and T-lymphocytes are the most important cells in the adaptive immune system. The adaptive immune system is the part that develops with age in contrast to the innate immune system, i.e. populations of neutrophils and macrophages. A significant difference between the two cell populations is that B-lymphocytes originate from the bone marrow, and the T-lymphocytes from the thymus gland. It is in these organs that the lymphocytes gain

their ability to differentiate between epitopes (i.e. a specific region of an antigen) that belong in the body and those that are foreign to the body. It is a common misconception that the lymphocyte maturation process is controlled by antigens when in reality it actually depends on the cells' genes. (Sjaastad et al. 2016)

After the maturation process, the lymphocytes begin to serve their purpose in the immune system as they begin circulating through the blood and lymphatic system, scanning for foreign epitopes. If a lymphocyte encounters the specific epitope it is coded to recognize it will start to proliferate and differentiate into effector lymphocytes, an example for B-lymphocytes being the antibody producing plasma cell and for T-lymphocytes the cytotoxic T cell. This process is known as activation and normally occurs in the body's secondary lymphoid organs. Another important difference is their function, B-lymphocytes being the only ones that can recognize antigens by themselves, leading to the production of antibodies towards specific epitopes. T-lymphocytes on the other hand must get the antigen presented to themselves by the cells' MHC surface proteins to form an immune response. For example, when cytotoxic T-cells are presented with an antigen, they are activated to fight intracellular microorganisms such as bacteria, viruses, and fungus as well as cancer cells. This cannot be done by the B-lymphocytes. (ibid.)

2.2 Canine malignant lymphoma

2.2.1 Etiology

The etiology of canine malignant lymphoma is at the moment not fully known, although there is a strong suspicion it might be multifactorial containing a strong genetic factor as significant differences in breed disposition have been noted. (Bennett et al. 2018)

Other factors as for instance: exposure to chemicals and magnetic fields as well as ionizing radiation may also play a significant part in the etiology. (Zandvliet 2016)

2.2.2 Clinical presentation and pathology

Lymphoma has been categorized into four distinctly separated groups depending on which organ system it originates from.

Multicentric lymphoma

In multicentric lymphoma, the cancerous lymphocytes originate from lymph nodes, which presents as a generalized lymphadenopathy with or without involving other organs, most commonly the spleen, liver, or bone marrow. This is by far the most common form of lymphoma as all other forms are considered rare. (Zandvliet 2016)

Mediastinal lymphoma

Mediastinal lymphoma means that the lymphocytes originate from the thymus gland and generally presents as thymic enlargement and mediastinal lymphadenopathy with or without involvement of the bone marrow. Dogs with mediastinal lymphoma more often present with dyspnea, a cough, dysphagia, and/or regurgitations, all caused by swelling of the mediastinal and sternal lymph nodes or thymus. (ibid.)

Alimentary lymphoma

In alimentary lymphoma the cancerous lymphocytes originate from the gastrointestinal tract's lymphoid tissue, MALT. It can be solitary, multifocal, or diffusely spread with or without involvement of regional lymph nodes. Alimentary lymphoma results in clinical signs related to the gastrointestinal tract such as frequent vomiting, diarrhea, inappetence, anorexia etc. (ibid.)

Extranodal lymphoma

Extranodal lymphoma can theoretically manifest in any part of the body. Examples of commonly affected organs are the spleen, liver, kidneys, eyes, pharynx, nasal cavity, skin and nervous system. Extranodal forms of lymphoma can theoretically give any clinical signs imaginable with the reasoning it can manifest in any organ. (ibid.)

Clinical application

Because of lymphoma's incredible diversity, it may be problematic to bundle all possible clinical signs under the diagnosis of "lymphoma". The clinical presentation will vary tremendously, and it is therefore wise to always relate the clinical signs to the specific form of lymphoma and their occurrence. For example, in a dog with general lymphadenopathy that also has a cough and trouble breathing, mediastinal lymphoma should be suspected. However, due to it being more common, regular multicentric lymphoma should still be the primary suspicion as it is more likely that enlarged mediastinal and bronchial lymph nodes are compromising the lungs. (ibid.)

Paraneoplastic syndrome

Paraneoplastic syndrome is common and defined as a line of nonspecific symptoms caused by the presence of a tumor in the body. The tumor produces signaling substances (i.e. cytokines, hormones, or mimicking substances) that leads to a variety of pathological consequences. (Zandlivet 2016)

A common paraneoplastic occurrence is that neoplastic cells are producing parathyroid hormone related protein (PTHrP), a protein that mimics the function of parathyroid hormone (PTH). This results in dogs getting hypercalcemia with the

following polyuria/polydipsia (PU/PD). According to Nelson & Couto (2020) hypercalcemia is seen in 20-40% of dogs with lymphoma and is most common in dogs with mediastinal lymphoma and lymphoma of T-lymphocyte origin. Hyperproteinemia is another paraneoplastic clinical sign that occurs as a result from a mono- or polyclonal gammopathy, in other words an abundant production of antibody components by B-lymphocytes. This sign, however, is quite uncommon. Anemia or thrombocytopenia occurs when neoplastic cells have infiltrated the blood system and are affecting the normal production of blood components by the bone marrow. (Nelson & Couto 2020)

Staging

Lymphoma is normally graded as low, intermediate, or high depending on the proliferative properties and size of the neoplastic lymphocytes. A stage of *I-V* is given to the dog depending on the manifestation and extent of the lymphoma (see table 1). (Nelson & Couto 2020)

A substage of *a* or *b* is then given to all patients, asymptomatic patients are substage *a*, and sick patients presenting with general clinical signs such as reduced general condition, fever, weight loss, vomiting and diarrhea, inappetence or anemia are substage *b*. (ibid.)

This whole process is commonly known as the staging process.

Table 1. Staging system for malignant lymphoma in dogs and cats.

Stage	Clinical findings
I	Singular affected lymph node
II	Several affected lymph nodes on one side of the diaphragm
III	Several affected lymph nodes on both sides of the diaphragm
IV	Hepato- or splenomegaly
V	Bone marrow or extranodal involvement

2.2.3 Diagnostic methods

Clinical presentation

As for most other diseases, very few dogs will be investigated for lymphoma unless there are any foregoing clinical signs. As mentioned earlier, generalized lymphadenopathy is the most common clinical sign of multicentric lymphoma. A general feeling of sickness often accompanies the dog alongside various changes in blood work. The clinical signs are often vague and elusive, which is why the clinician

should always consider lymphoma as a differential in these cases. (Nelson & Couto 2020)

Cytology and PARR

A definitive diagnosis is achieved through cytologic evaluation most commonly from a fine needle aspirate (FNA). According to the personal experiences of Nelson and Couto (2020), this is possible in 90% of dogs with lymphoma, in the remaining 10% diagnosis is achieved through histopathology, flow cytometry or molecular techniques such as immunohistochemistry or immunocytochemistry, in other words techniques using antibodies to characterize a population of cells.

PCR for antigen receptor rearrangement (PARR) is a diagnostic technique used "to determine whether the lymphocytes in a specimen share identical antigen receptors (i.e. are "clonal"), or whether they are genetically different (i.e. are "polyclonal")". This technique can be used on the same cytology slide taken by the FNA and heightens the sensitivity for detecting lymphoma as a large population of lymphocytes sharing the same antigen receptor are highly suggestive of lymphoma. (VDx Veterinary Diagnostics and Preclinical Research Services 2021)

PARR has also been shown by Aresu et al. (2014) to be a useful tool in the follow up diagnostic process of lymphoma to determine the risk of relapse of dogs in remission by monitoring minimal residual disease.

Blood samples

Analyzing blood samples is not diagnostic in itself, although, they do aid the clinician in staging and getting a wider picture of the patient's disease. A varying array of changes can be seen in the hematology, for instance a non-regenerative anemia as a consequence of bone marrow suppression, varying forms of leukocytosis (neutrophilia, monocytosis and eosinophilia), thrombocytopenia and different forms of cytopenias. A lymphocytosis would be expected, but since the lymphocytes change their appearance, they are miscategorized as monocytes and that is why a monocytosis is often observed instead. This however does not rule out the possibility of seeing a lymphocytosis as most hematological changes are possible. Common biochemical parameters are hypercalcemia and gammopathies (i.e. excessive production of antibody components). Hyperproteinemia is uncommon but may be seen alongside a gammopathy. (Nelson & Couto 2020)

Diagnostic imaging

Imaging is often included in the diagnostic process of lymphoma to visualize anatomical and topographical changes. These changes are most commonly seen in the thoracic and abdominal cavity, giving the clinician an idea of how spread the neoplasm is. The most common radiological changes seen are general enlargement of intrathoracic and intraabdominal lymph nodes and other organs, spleen and liver foremost. X-ray or CT are the preferred methods to diagnose mediastinal lym-

phoma. Ultrasonography is routinely used for diagnosing alimentary lymphoma and also provides a means of retrieving a FNA or biopsy for the definitive diagnosis. (Nelson & Couto 2020)

2.2.4 Treatment

Chemotherapy

Chemotherapy is the cornerstone in treating lymphoma in dogs as it is a systemic neoplasia and almost always will become systemic even though it might be local initially. It puts 80-90% of dogs into remission for 8-16 months, which is a lot longer than the estimated total survival time of 4-8 weeks in untreated dogs. (Nelson & Couto 2020)

The most common protocols of chemotherapy are the COP-protocol, which include cyclophosphamide (immunosuppressant that interferes with DNA and RNA production), vincristine (alkaloid that inhibits cell division), prednisolone (corticosteroid) and the CHOP-protocol, which also includes doxorubicin (anti-biotic that block enzymes necessary for DNA-replication and transcription). Worthy to mention, there has been evidence that prednisone may be left out of these first-line multidrug protocols without any significant difference being observed by Zandvliet et al. (2013). Due to the fact that these protocols are quite time and money consuming, alternatives for lower cost and effort are available. For example a protocol described by Al-Nadaf et al. (2018) including only doxorubicin and prednisone alone with a longer interval between dose administration than the standard protocols.

L-asparaginase is an enzyme that breaks down the non-essential amino acid asparagine, which the lymphoma cells cannot produce on their own but instead rely on circulating asparagine, which they need to survive. It can be added to a protocol, for example to intensify the COP- and CHOP-protocols (in that case called L-COP and L-CHOP). L-asparaginase has also shown to play a part in certain situations of treatment, for instance Nakagawa et al. (2021) studied continuous administration in dogs with presumed alimentary lymphoma. Cawley et al. (2020) examined L-asparaginase in combination with other cytostatic agents to treat relapsed multi-centric lymphoma and found this to be effective.

In some older studies by Jeffreys et al (2005) and MacDonald et al. (2005) L-asparaginase was found to not influence clinical remission or median overall survival time. On the other hand, Valerius et al. (1997) found in a previous study L-asparaginase to improve the initial response rate to chemotherapy, which the authors deemed as having some significant clinical implication.

The protocol most frequently used at UDS is a modified L-CHOP protocol called Adria-Plus containing L-asparaginase, dexamethasone, prednisolone, doxorubicin, chlorambucil, cyclophosphamide and hydroxyurea. (Piek et al. 1999)

In case a dog fails to respond to a first-line cytostatic protocol or in case of relapse, a rescue protocol might be of great help though it rarely has the same impact on survival as the first-line protocol. (Zandvliet 2016)

Surgery, radiation therapy and bone marrow transplantation

The patient might benefit from surgical resection of local lesions, radiation therapy or bone marrow transplantation. However, this is decided upon an individual basis and only if it is deemed to prolong remission or enhance prognosis.

Splenectomy is an example of a surgical intervention that may drastically improve prognosis of survival in cases of splenic lymphoma where the neoplasia is still restricted to the spleen only. (van Stee et al. 2015)

Radiation therapy has shown some promising results in combination with chemotherapy, but it is evident that more research on the area is needed for this treatment option to become part of a standard procedure. (Lurie et al. 2009; Williams et al. 2004)

In the field of bone marrow transplantation there is little convincing data as it still seems to be in the start-up. There have been some promising pilot studies, but it is still too soon to draw any conclusions. (Thamm 2019)

Immunotherapy

The use of immunologic therapies seems to be the next step in the progression of treating lymphoma in dogs. Monoclonal antibodies, various lymphoma vaccines, T-cell based therapies and the use of “small molecules” are currently being researched in order to improve treatment outcomes. Some promising results have been observed but there is still a long way to go in order for such methods to be implicated into standard treatment options. (Thamm 2019)

2.3 Risk assessment and prognostication

2.3.1 Demographic risk factors

It has long been discussed which demographic factors of risk that may prime for the development of lymphoma. It is known that some breeds are more prone to becoming sick than others, for example the Golden Retriever, Labrador Retriever, Bullmastiff, Rottweiler, and Boxer to name a few, shown by Bennet et al. (2018). They also found male dogs to have an increased risk compared to bitches alongside gonadectomized dogs independently of sex. Grüntzig et al. (2016), Villamil et al. (2010) and Zink et al. (2014) also found gonadectomized male dogs to possess an increased risk of falling ill.

One theory by Kutzler (2020) as to why gonadectomized dogs are more often affected by malignant lymphoma, is that luteinizing hormone triggers lymphocytes and lymphoma tissue to proliferate.

Another study by Kiupel et al. (1999), who originally studied proliferative markers of cancer cells called AgNORs, found these to be prognostic for both total survival time and disease-free survival. He also found age, weight, sex, and stage not having any prognostic value.

Dogs that live near industrial areas or areas that on a regular basis are exposed to chemicals, magnetic fields, or ionizing radiation, have also shown a higher incidence than the mean population of dogs. (Zandvliet 2016)

2.3.2 Prognostic factors

Prognostic factors include which form of lymphoma has affected the dog, for instance multicentric lymphoma has a better prognosis for total survival time than mediastinal, alimentary, and extranodal lymphoma depending on the affected organ. Immunophenotype, grade, stage and substage also have an impact. Lymphoma of B-cell origin generally has a better prognosis than T-cell lymphoma, low grade having a better prognosis than intermediate-high grade, stage *I-IV* better than *V* and substage *a* better than substage *b*. (Nelson & Couto 2020; Zandvliet 2016)

A study by Huang et al. (2021) showed that signs of paraneoplastic syndrome are worsening the prognosis. Anemia was also associated with a shorter progression-free interval after treatment and thrombocytopenia with a longer survival time.

Blood cell ratios have been found to possibly possess some prognostic value, but it is evident that more research on the area is needed as several non-oncological factors may also influence the results. (Henriques et al. 2021; Tagawa et al. 2019)

Biomarkers, more specifically the TK-1 biomarker have been analyzed for its prognostic value, but different authors have presented conflicting results. Von Euler et al. (2004) claims it is prognostic while Falk (2018) and Zaidi et al. (2023) claims the contrary. TK-1 has also been found by Saellström et al. (2022) to be useful for prognostication in a biomarker panel for lymphoma in combination with CRP.

Dogs not treated with corticosteroids prior to chemotherapy have been shown by Marconato et al. (2011) to have a higher probability of long-time survival and by Gavazza et al. (2008), a longer overall survival time. Price et al. (1991) found that dogs that had received treatment with corticosteroids before chemotherapy showed a shorter remission time than untreated dogs before chemotherapy.

How well a dog responds to chemotherapy also seems to have some prognostic value for survival as dogs that showed minimal residual disease (MRD) had longer progression-free survival (PFS) compared to dogs that showed signs of residual disease. (Sato et al. 2013)

3. Material and method

3.1 Study configuration and study population

A single-center retrospective medical record study was carried out using medical records from the 356 dogs and were diagnosed with lymphoma, either as a primary or as a secondary diagnosis that visited UDS between 2005-2021. Examples of occasions when lymphoma was set as a secondary diagnosis were dogs that visited for another primary purpose and lymphoma coincidentally was diagnosed along the way or dogs that had their diagnosis of lymphoma since earlier and now visited the hospital for another reason.

3.1.1 Definition of variables

From the medical records, data was collected on demographics, pathology, paraneoplastic clinical signs, and treatment. Protection of personal data was ensured as the Swedish University of Agricultural Sciences (SLU) has adequate protocols to uphold GDPR which, includes the use of medical records for research purposes. In addition, no personal data on the owners was collected. The demographic parameters included: sex (*male/female*), age at onset of first clinical sign of lymphoma (*years*), weight (*kg*), breed, and whether the dog was gonadectomized or not (*yes/no*).

Regarding the specific pathology of the lymphoma, data was collected on: survival time after onset of first clinical sign of lymphoma (*days*), form of lymphoma (*multicentric/mediastinal/alimentary/extranodal*), immunophenotype (*B-/T-lymphocytic origin*), size of neoplastic lymphocytes (*small/intermediate-large*), stage (*I/II/III/IV/V*), and substage prior to treatment (*a/b*).

Data on paraneoplastic clinical signs prior to treatment included: anemia (*yes/no*), hypercalcemia (*yes/no*), and hyperproteinemia (*yes/no*). To clarify, thrombocytopenia was not included as a sign of paraneoplastic syndrome in this study.

Lastly, variables regarding treatment included: treatment with cytostatic agents (*yes/no*) and if the dog was left untreated (*yes/no*) (i.e. dogs that were either euthanized at diagnosis or dogs that received only symptomatic treatment until euthanization or death). To clarify, treatment with only L-asparaginase was categorized as cytostatic treatment even if only given as a single injection.

In some cases of missing data, certain parameters of individual dogs were left out, meaning they could still provide some statistical data for other variables. In other cases of missing data, assumptions could be made from anamnestic information. For example, if the owner did not know the exact date their dog fell ill

but said: “somewhere around a month ago”, one month prior to the consultation was assumed to be the exact date the dog fell ill.

3.2 Statistical analysis

For the statistical analysis, the statistical program Minitab was used. The adapted approach to analyzing the data was to initially make Kaplan-Meier estimates accompanied by both a Log-Rank-test and a Wilcoxon-test. The variables that were statistically significant in the Kaplan-Meier were chosen to be included in a Cox Regression where statistically nonsignificant variables were excluded until the Goodness-of-Fit test deemed the model suitable. For this, some variables were pooled, for example stage (*I-IV/V*), one or more signs of paraneoplastic syndrome (*yes/no*), age (*<7.6 years/>7.6 years*), and weight (*<27.3kg/>27.3kg*), thus making them categorical variables instead of continuous or easier to censor out missing values and making the dataset more complete. 279 dogs remained after the sorting of the dataset.

All statistical tests were performed at a confidence interval of 95%.

3.2.1 Data Modulating

Dogs that had an unknown time of death or were euthanized because of some concurrent illness were considered lost-to-follow-up and thereby censored. Dogs that only had a survival time of maximum one day were also censored as all of these were euthanized upon diagnosis.

After data collection, it was found that data could be easier to manage if variables were pooled as it meant missing values could be filled in and variables containing few values could still be included in the analysis. A great example of this is the signs of paraneoplastic syndrome variable that was pooled from the anemia, hypercalcemia, and hyperproteinemia variables, which all three contained quite a lot of missing values.

3.3 Literature search

A thorough review of the literature was initially made using the database Scopus using a search phrase including the words *Dog*, *Dogs*, *Canine*, and *Lymphoma* in combination with either *Risk* or *Prognosis*. An initial list of relevant articles and review articles was made and subsequently the same search phrase was used on PubMed to complement the literature search. Reference lists of relevant review articles were also examined to widen the list of relevant articles and studies that could potentially be included in this study.

4. Results

4.1 Demographic information

The sorting of patients on malignant lymphoma as primary or secondary diagnosis resulted in 356 dogs between the years 2005 and 2021.

The mean age at sign of first clinical sign was 7.6 years (n=356) around which the dogs were normally distributed. The mean weight was 27.3 kg (n=333), the proportion of dogs weighing over 20 kg (this was decided to represent heavy dogs) being 69.1%.

Mixed breed was the most frequently occurring type of dog (n=62, 17.6%), second most common was Golden Retriever (n=28, 7.9%), third most common Rottweiler (n=23, 6.5%), fourth most common German Shepherd (n=12, 3.4%), fifth most common Miniature Schnauzer and Border Collie (n=10, 2.8%) respectively.

In total, 160 (44.9%) of the dogs were female and 196 (55.1%) dogs were male (n=356), of which 88 were castrated (24.7%).

4.2 Pathological information and treatment

In total, 289 multicentric (83.8%), 14 mediastinal (4.1%), 19 alimentary (5.5%), and 23 extranodal lymphomas (6.7%) were diagnosed, 44 (55.7%) of which were of B-lymphocyte origin and 35 (44.3%) of T-lymphocyte origin. Twelve (7.7%) of the lymphomas were small cell lymphomas and 143 (92.3%) were of intermediate-large cell types. In total, 99 (29.2%) dogs did not show any signs of systemic illness upon diagnosis (a-symptoms) but 240 (70.8%) did (b-symptoms).

Stage *I* and *II* were uncommon with four (1.2%) and 15 (4.4%) dogs in each respective group. Stage *III*, *IV*, and *V* contained the majority of the dogs with 110 (32.5%), 75 (22.2%) and 134 (39.6%) in each group respectively.

There were 92 (33.8%) dogs that showed hematological signs of anemia and 180 (66.2%) that did not, 19 (8.6%) dogs had elevated levels of calcium in their blood and 203 (91.4%) that did not. Lastly, six (3.4%) dogs had elevated levels of protein in their blood and 168 (96.6%) that did not. As these three variables contained many missing values their groups were pooled into a group where 111 (39.8%) showed at least one sign of paraneoplastic syndrome and 168 (60.2%) that did not.

In total, 209 (61.3%) did receive treatment with chemotherapy and 132 (38.7%) that did not. At last, 92 (27.0%) dogs did not receive any treatment at all for their

lymphoma except for symptomatic treatment or euthanasia, meaning 40 (11.7%) dogs received treatment with solely corticosteroids.

4.3 Kaplan-Meier estimates

From the Kaplan-Meier analysis it was concluded that form, stage, substage, signs of paraneoplastic syndrome, chemotherapy and no treatment seemed to contain statistical significance for survival time (see table 2). All but chemotherapy were included in the Cox Regression as it was decided that it would be stratified by dogs that received treatment or not, thus excluding the chemotherapy variable in the untreated group.

Another decision was to use the pooled group for stage as it was deemed unsuitable to include the stand-alone groups for stage *I* (n=2) and *II* (n=6) as they included such few uncensored values. Also, the pooled group for weight (n=198) was included, even though the p-value for the Log-Rank test exceeded 0.05, the reason being the group containing quite a lot of values.

Table 2. p-values and censoring information from Kaplan-Meier estimates.

Variable (the percentage within brackets represent the proportion of dogs that had available information for each respective variable)	n (Uncensored)	n (Censored)	Log-Rank	Wilcoxon
Pooled age (*<7.6 years/>7.6 years)* (100%)	104/104	66/75	p=0.902	p=0.805
Pooled weight *(<27.3 kg/>27.3 kg*) (93,5%)	98/100	76/57	p=0.090	p=0.040
Sex (*male/female*) (100%)	112/96	79/62	p=0.693	p=0.767
Castration status (*yes/no*) (100%)	55/153	33/108	p=0.784	p=0.714
Form (*multicentric/mediastinal/alimentary/extranodal*) (96,9%)	170/10/12/14	118/4/7/9	p=0.003	p=0.001
Immunophenotype (*B-/T-lymphocytes*) (22,2%)	24/23	20/12	p=0.059	p=0.100
Neoplastic cell size (*small/intermediate-large*) (43,5%)	8/96	4/47	p=0.042	p=0.080

Stage (*I/II/III/IV/V*) (94,9%)	2/6/62/43/90	2/9/48/31/44	p=0.001	p=0.000
Pooled stage (*I-IV/V*) (94,9%)	112/90	90/44	p=0.001	p=0.000
Substage (*a/b*) (95,2%)	53/149	45/90	p=0.002	p=0.000
Anemia (*yes/no*) (76,4%)	59/104	33/75	p=0.204	p=0.008
Hypercalcemia (*yes/no*) (62,4%)	13/117	6/85	p=0.021	p=0.072
Hyperproteinemia (*yes/no*) (48,9%)	5/99	1/69	p=0.236	p=0.642
Signs of paraneoplastic syndrome (*yes/no*) (78,4%)	73/95	38/72	p=0.036	p=0.002
Received treatment with chemotherapy (*yes/no*) (95,8%)	113/91	96/39	p=0.000	p=0.000
Received no treatment for lymphoma (*yes/no*) (95,8%)	71/133	20/115	p=0.000	p=0.000

4.3.1 Kaplan-Meier-curves

Below are the curves obtained from the Kaplan-Meier analysis, displaying the differences in proportions of dogs surviving for form of lymphoma and stage (see figure 1), substage and signs of paraneoplastic syndrome (see figure 2), and dogs treated with chemotherapy and untreated dogs (see figure 3).

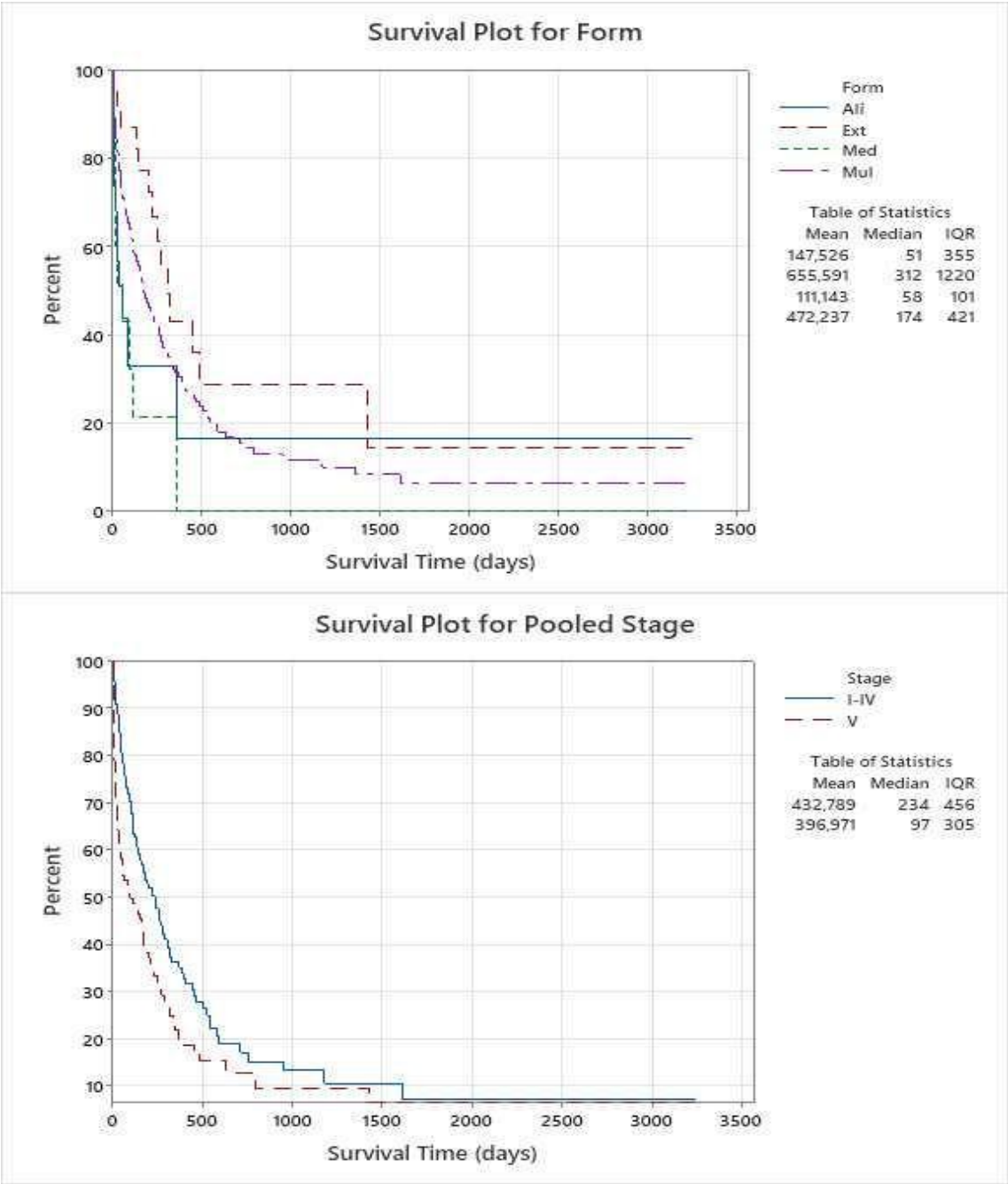

Figure 1. Kaplan-Meier-curves of form of lymphoma and pooled stage.

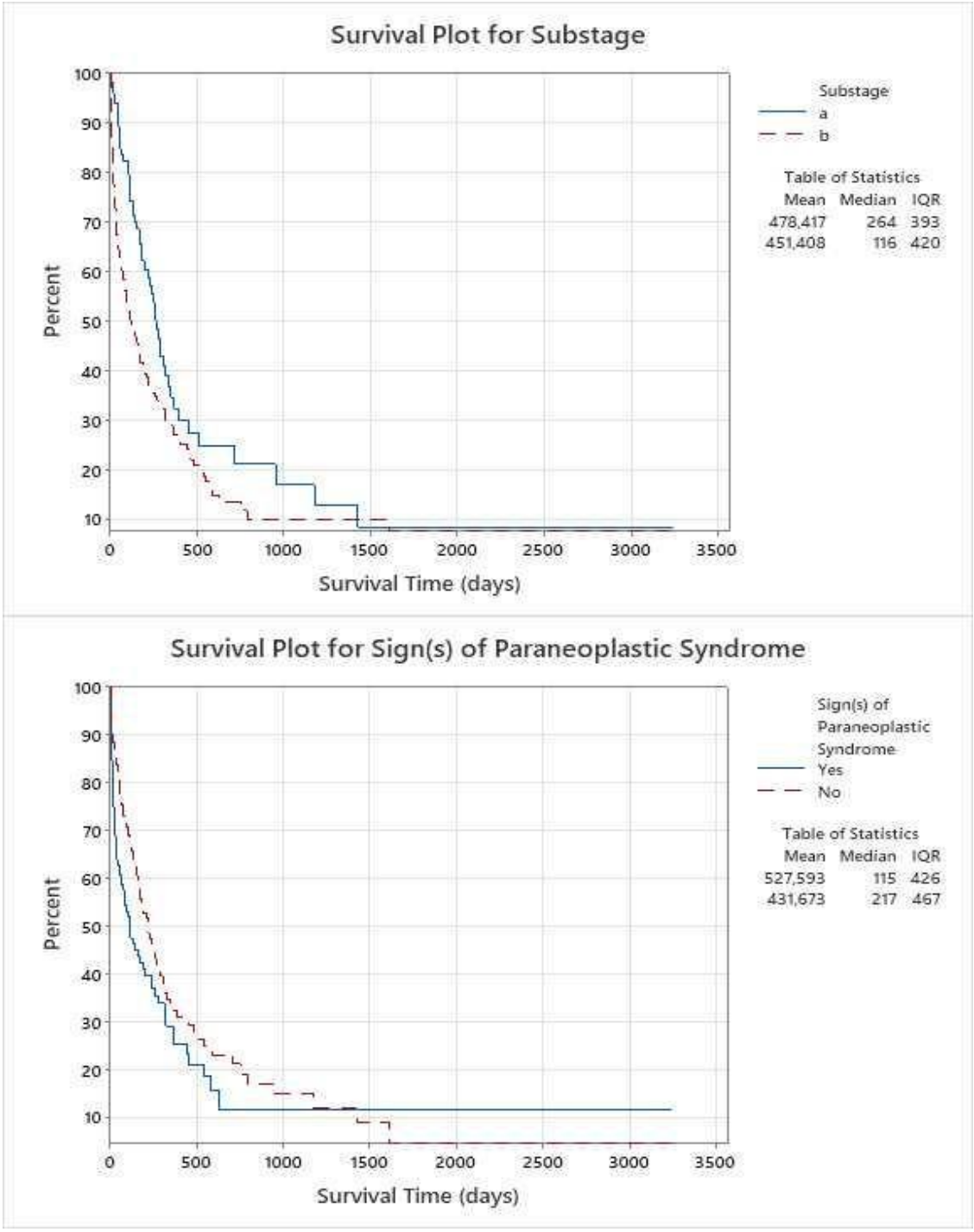

Figure 2. Kaplan-Meier-curves of substage and sign(s) of paraneoplastic syndrome.

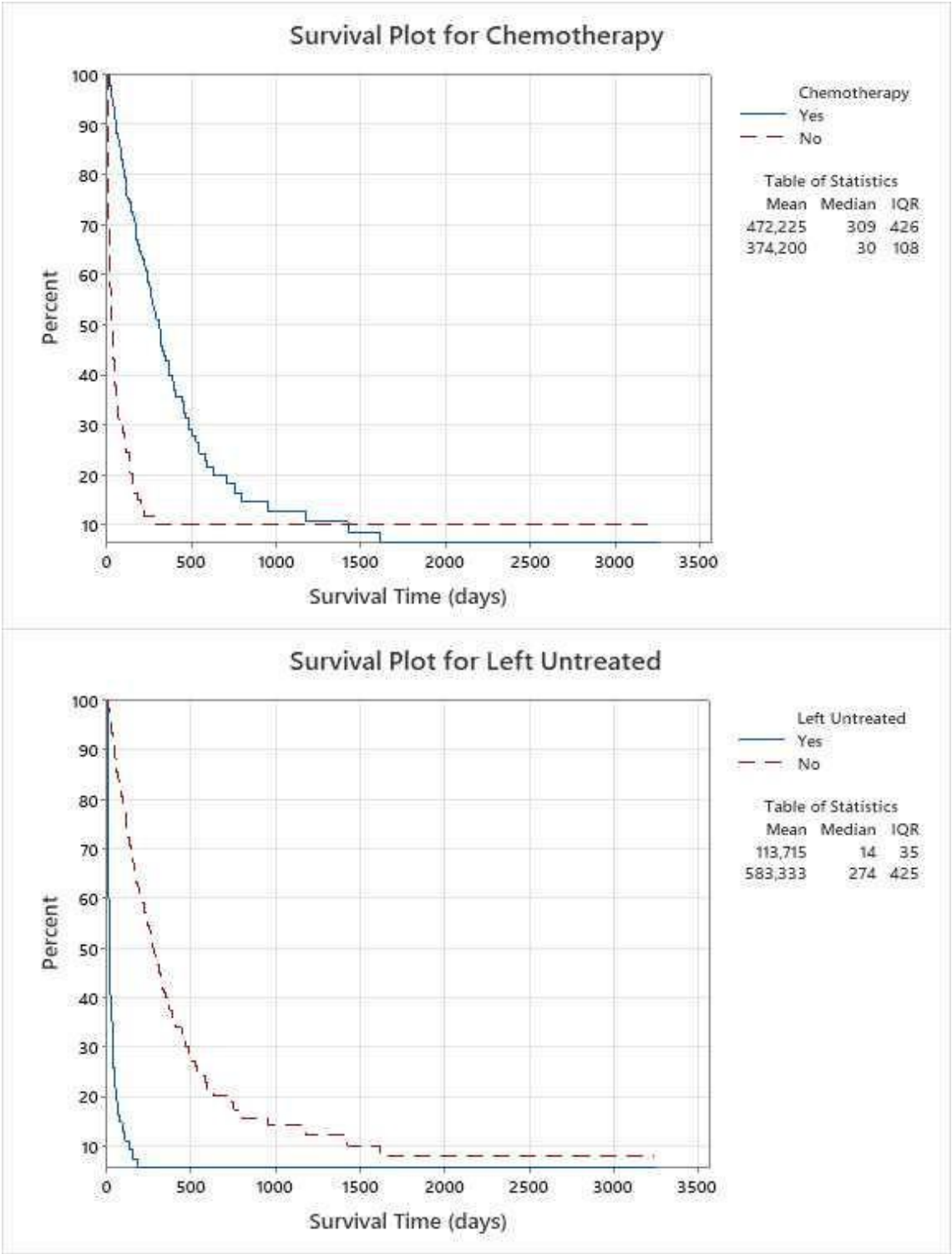

Figure 3. Kaplan-Meier-curves of dogs that received chemotherapy and untreated dogs.

4.4 Cox regression

Once the Goodness-of-Fit test of the Cox Regression reached p-values below 0.05 only form (p=0.061) and pooled stage (p=0.010) remained as variables of the ANOVA-table (see figure 4).

Stage *V* patients showed a relative risk of 1.6 relative to stage *I-IV* (see figure 5) for a shortened survival time. Mediastinal lymphoma showed a relative risk of 2.7 towards extranodal form and multicentric form a relative risk of 2.3 in relation to extranodal form. The remainder of categorical variables displayed a 95% CI overlapping 1.

Analysis of Variance

		Wald Test	
Source	DF	Chi-Square	P-Value
Form	3	7,37	0,061
Stage2	1	6,73	0,010

Figure 4. ANOVA table from the Cox Regression.

Relative Risks for Categorical Predictors

		Relative	
Level A	Level B	Risk	95% CI
Form			
Ext	Ali	0,6137	(0,2620; 1,4376)
Med	Ali	1,6702	(0,6665; 4,1858)
Mul	Ali	1,3951	(0,7144; 2,7246)
Med	Ext	2,7214	(1,0905; 6,7917)
Mul	Ext	2,2732	(1,1884; 4,3481)
Mul	Med	0,8353	(0,3951; 1,7660)
Stage2			
V	I-IV	1,6283	(1,1265; 2,3537)

Risk for level A relative to level B

Figure 5. Relative risk table from the Cox Regression.

5. Discussion

The discussion will compare the results from this study with the current knowledge concerning canine lymphoma as well as describing the limitation with this retrospective study based on medical records from a single-center.

5.1 Prognostication

None of the variables displayed a relative risk-value less than one and a confidence interval that did not include one simultaneously, hence is why no variable was considered a positive prognostic factor in the Cox Regression.

From what Zandvliet (2016) has compiled, it is favorable for survival and remission to be a "female, small-breed dog with stages *I-IV* and substage *a*" rather than a "male, large-breed, stage *V*, and substage *b* dog".

5.1.1 Population characteristics and variables

The mean age, weight and sex distribution of the dogs suggest older male dogs of larger breeds being at increased risk of falling ill. Interestingly, neither the Kaplan-Meier estimation nor the Cox Regression found any of these variables to impact survival time, which is consistent with what Kiupel et al. (1999) found. However, Keller et al (1993) did find sex and Garrett et al. (2002) found weight to significantly impact survival so it can be concluded that there are evident differences in our results.

The proportion of Golden Retrievers and Rottweilers also suggests some breed predisposition to be at play here as was mentioned previously in the literature review. This is interesting as Golden Retrievers were in the baseline category for tumor associated deaths in an extensive survey done by Egenvall et al. (2005) who examined cause-specific mortality in a population of over 350,000 insured Swedish dogs. They also mention that Golden Retrievers accounted for: "more than 6% of the population". Assuming the breed has not grown in popularity in recent years, this could imply that Golden Retrievers were overrepresented in this study and fortifies the claim that the etiology contains some genetic component.

Variables regarding characteristics of the dogs (sex, age, weight, castration status and breed) were the easiest to find as good as complete data on in contrast to for example immunophenotype and neoplastic cell size. One major factor for this was that immunophenotype and neoplastic cell size often were confined to attached files in the medical records that could not be retrieved, as the medical records had

been downloaded as PDF-files and thereby invalidating the links. As was shown by the Kaplan-Meier-analysis, these variables were not significant enough to be included in the Cox Regression. However, more values would have been useful in order to strengthen this decision.

One variable that would have been interesting to include is thrombocytopenia as it subjectively was found to be a frequently discussed feature during the staging process. Another thought that struck while discussing the results was if dogs that presented with more than one sign of paraneoplastic syndrome would have a poorer prognosis than dogs with only a single sign? This could be done with the information gathered in this study as it is easy to see if a dog showed multiple signs of paraneoplastic syndrome or not in the datasheet. However, due to time restrictions, this was not possible after the statistical analysis had already been executed.

5.1.2 Type of lymphoma

To start off with, a p-value of 0.061 does not suggest that the relative risk-values for the forms of lymphoma are statistically significant. However, the results will still be discussed as the goodness-of-fit test deemed the analysis suitable when form was included. It also calls for some interesting reflections.

Both multicentric and mediastinal lymphoma had an increased risk of shortening the survival time when compared to extranodal form in this study. This seems to fall in line with the literature in the case of the mediastinal form but not for the multicentric form, which is supposed to inform the patient of a more promising prognosis. However, the confidence interval informs that the relative risk could be as low as 1.2 and the fact that only 14 uncensored values of the extranodal form were enrolled in the statistical analysis heightens the uncertainty in these results.

In this study, extranodal form included cutaneous lymphoma, which Fontaine et al. (2010) found could have a median time of five months between onset of the disease and the diagnosis. This could explain a part of this result as the survival time was measured from onset of the disease and the multicentric form seldom had a history quite that long.

Another surprising observation was that alimentary lymphoma did not show any increased risk relative to any other form, not even the multicentric form. Frank et al. (2007) showed an example of how poor the prognosis of alimentary lymphoma mostly is with a median survival time of 13 days. A second example of its grave prognosis was presented by Rassnick et al. (2009) who found 50% of dogs with alimentary lymphoma achieving complete remission for a median of only 86 days after chemotherapy.

Zandvliet (2016) says: “despite this generalization, it has to be realized that within all of these groups, subsets of dogs may perform better”.

5.1.3 Stage

This study showed, as the literature suggested, that being a stage *V* patient did worsen the prognosis for survival relative to being a stage *I-IV* patient. This seems reasonable as the criteria for being a stage *V* patient is to have circulating neoplastic lymphocytes present in the blood, hence it should be easier for them to manifest in any part of the body and thereby worsening the prognosis.

5.1.4 Substage

The Kaplan-Meier estimation did show results consistent with the literature. However, substage did not stay significant for total survival time in the Cox Regression, which is surprising as substage is one of the most consistent prognosticators for survival time and/or disease-free interval. (Keller et al. 1993; Garrett et al. 2002)

One theory as to why this result was obtained is that dogs could have been miscategorized as substage *b* in cases when the owner is awaiting spontaneous recovery. Had the dog visited earlier it may not have had time to migrate from substage *a* to *b*.

Missing anamnestic information, for example blood work or x-rays from the referral veterinarian could also explain some part as to why dogs could be miscategorized as *a* instead of *b*. Say the hematology showed an anemia that was not included in the referral.

5.1.5 Signs of paraneoplastic syndrome

The information on blood parameters were quite inconsistent, possibly due to referral veterinarians not including lab results or not sustaining adequate diagnostic procedures for lymphoma, the data for these variables were pooled into a group called: “one or more sign(s) of paraneoplastic syndrome”. The results are hard to interpret as the data held up in the Kaplan-Meier analysis but not in the Cox Regression, hence is why it is deemed that more research is needed preferably with the paraneoplastic syndrome of lymphoma patients as the main objective.

The proportion of dogs that actually had elevated levels of calcium in their blood was quite a bit smaller than the proportion that Nelson and Couto (2020) stated (20-40%). Zandvliet (2016) does however, mention that 10-15% of dogs with lymphoma experience hypercalcemia. This is backed up further by Rosol et al. (1992) who found 8.3% of dogs with lymphoma to have increased levels of PTHrP.

Anemia was the most common paraneoplastic sign and frankly, quite common as every third dog out of the 272 dogs with available information on this variable, had anemia of some degree. CBC was also the most frequently analyzed variable and anemia could theoretically have been included as a stand-alone variable in the

Cox Regression as it has been proven earlier by Abbo & Lucroy (2007) and Miller et al. (2009) that anemia has a significant impact on survival time. This, however, was not preformed as it was decided wiser to use the pooled group of paraneoplastic signs.

The proportion of 3.4% of dogs with hyperproteinemia is supposedly low. However, proper research on the general prevalence of elevated protein levels in blood from dogs with lymphoma seems to be missing and therefore it is difficult to verify these results.

5.1.6 Chemotherapy

It is common knowledge that chemotherapy is the golden standard of treating lymphoma today as the future immunologic treatment options still are a bit out of reach both economically for the owner and research wise for the veterinarian. It is therefore not surprising that the dogs that received chemotherapy survived longer than those that did not, clearly exhibited by the Kaplan-Meier-curve. Zandvliet (2016) writes: "a complete remission is obtained for most dogs and lasts for a median period of 7-10 months, resulting in a median survival of 10-14 months". It is complicated to compare this information to the Kaplan-Meier-curve as it is difficult to know how big of a proportion "most" actually is, in combination with the evident confounding bias in this study which is discussed further down.

5.1.7 Untreated dogs

As for the chemotherapy variable, it is not surprising that dogs that received treatment other than symptomatic treatment for their lymphoma survived longer than those that did not. It should however be pointed out that only dogs that had a survival time of one day or less were censored due to an unreliable survival time. This meaning, dogs that had a survival time of as low as two days were included in the statistical analysis even though, through sheer biological reasoning, it can be concluded that two days is not a reasonable value of true survival time. The reason for the decision to censor just these patients was to avoid an arbitrary limit for survival time and instead conduct an upfront discussion about this interference.

5.2 Limitations

It was brought up in the previous discussion that the study has its limitations, and the results should be interpreted with care.

There is evident confounding bias as owners are not always willing or have the opportunity to treat their dogs with chemotherapy. Examples of confounders are financial limitations as a full chemotherapy protocol costs a considerable amount

and social situations, most commonly small children in the home that might not be able to distinguish from the dog's excrement.

Below are the general limitations that follow from a retrospective study based on medical records.

Some variables were based partially on anamnestic information, for example survival time, recall bias must be considered in this case, as owners might not memorize the exact date their dog fell ill (this would also impact age at onset of the disease). As for substage, dogs could have been miscategorized as substage *b* instead of *a* due to the Clever Hans effect, owners may have claimed their dog had been feeling a bit down when they, in reality, had not done so. Why this occurs is difficult to answer, maybe the owner is flabbergasted after hearing their dog might have cancer.

The number of missing values of some variables does heighten the uncertainty of some results. It would have been preferable to gather more values of immunophenotype and small cell lymphomas in order to get a better grip of their respective prognostic contributions, maybe this could be a project of the future. Same goes for all forms except for multicentric, which managed to provide quite a substantial number of values. The reader must take into consideration the lack of these values.

Lastly, I deem it wise to limit the applications of these results to UDS and their clientele as no geographical data was collected due to time limitations, hence it is crucial to consider possible differences in approach to lymphoma between hospitals. In other words, it is important to remember that these results best describe the population of dogs that visit UDS.

5.3 Conclusion

The purpose of this retrospective study was to hopefully be able to draw conclusions from medical records of dogs diagnosed with lymphoma about prognostication of survival time. Multicentric and mediastinal lymphoma were associated with shorter survival compared to extranodal lymphoma. This result should, however, be handled with care as multicentric lymphoma is not known for having a poorer prognosis than extranodal form. Stage *V* was also shown to have a worse prognosis than stage *I-IV*, which seems reasonable as it has been shown many times before.

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Popular science summary

Canine malignant lymphoma is a common neoplasm of the dog that originates from specific white blood cells that normally defend the body against disease, meaning they become very sick as these cells can no longer help the body fight off pathogens such as bacteria, viruses, fungi, and cancer cells. Lymphoma can manifest in different parts of the body; the lymphatic system (multicentric form), the digestive tract (alimentary form), the thoracic cavity (mediastinal form) or in some other organ or part of the body, for example the skin och nervous system (extranodal form). Some very common clinical signs are generalized swelling of lymph nodes accompanied by a general feeling of sickness, which is often also the cause of detection. An intricate diagnostic process called staging is carried out in order to best decide what treatment the dog is eligible for and to prognosticate the chance of survival. This includes blood samples, x-ray, and evaluation of the neoplastic cells under a microscope, and results in a stage of I-V.

What causes canine lymphoma is currently not fully known. There are suspicions of several factors interplaying such as genetic factors and exposure to radiation, chemicals, and magnetic fields. Examples of supposedly genetic factors are type of breed (Golden Retrievers and Rottweilers seem to be affected more often than other dog breeds), sex and weight. It is believed that large castrated male dogs are at increased risk of being affected.

Lymphoma in dogs is treatable by chemotherapy or steroids but the treatment is rarely curative as to why a lot of effort must be put into assuring quality of life between treatment sessions. This is where prognostication comes into play as the veterinarian uses certain signs to maneuver the prognosis and make a crucial decision whether to keep treating or to euthanize.

This study aimed to analyze what prognostic factors may apply to dogs that visit the University Animal Hospital (UDS) at Swedish University of Agricultural Sciences (SLU) and whether they differ from the general idea scientists may have of the disease.

The data was collected from medical records of all dogs that visited UDS between 2005-2021 and were diagnosed with canine malignant lymphoma. This is interesting as it is a widely known phenomenon that results from similarly deducted studies may differ between different sources. For example, if this exact same study would be performed at two or more different hospitals, all of them would probably be presented with different results, maybe similar but definitely not the exact same. This is why studies such as this one are of interest to carry out as it gives the veterinarians at the hospital information about the patients visiting precisely their hospital.

It was found that the form of lymphoma, stage and substage (if the dog experienced general signs of illness or not) has significant prognostic properties. It was also found that dogs that received treatment with chemotherapy managed better than those that received treatment with steroids only, that managed better than those that received no treatment at all. Dogs with lymphoma in their lymphatic system or thoracic cavity had a 2.3 and 2.7 times higher risk of having a shorter survival time than dogs with lymphoma in any other part of the body except for the digestive tract respectively. It was also shown that dogs that were stage V had a 1.6 times higher risk of living shorter than those with stage I-IV.

All forms of lymphoma, except for the multicentric one, are quite rare, hence is why few dogs with these forms were included in the study. In order to ascertain more precise results regarding these forms more research is needed in the future.

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