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# **Evaluation of platelet indices as prognostic factors in canine lymphoma**

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## Contents

1. Introduction .....	1
2. Literature review.....	2
2.1. An overview of canine lymphoma.....	2
2.1.1. Clinical signs of canine lymphoma .....	2
2.1.2. Diagnosing canine lymphoma .....	3
2.1.3. Treatment and prognosis of canine lymphoma.....	5
2.2. An overview of platelets.....	8
2.2.1. Platelets significance in disease in general.....	9
2.2.2. Platelets and cancer .....	9
2.2.3. Lymphomas effect on platelets.....	10
3. Goals and hypothesis .....	11
4. Materials and methods.....	12
4.1. Patients .....	12
4.1.1. Patient selection.....	12
4.1.2. Patient characteristics .....	12
4.1.3. Diagnostic methods .....	13
4.1.3.1. Lymph node and bone marrow samples .....	13
4.1.3.2. Histopathology and immunohistochemistry.....	14
4.1.3.3. Immunophenotype determination by flow cytometry .....	14
4.1.4. Treatment methods .....	15
4.1.5. Assessment of remission, relapse, and survival data.....	15
4.2. Reference population.....	17
4.3. Blood samples and platelet analyses .....	17
4.4. Statistical analyses.....	18
5. Results .....	18
5.1. Eligible patients .....	18
5.1.1. Patient characteristics .....	19
5.1.2. Tumor characteristics of the patients.....	19
5.1.3. Treatment and survival of the patients .....	20
5.2. Platelet indices of the patients .....	24
5.2.1. Comparison of PLT, PCT and MPV to a healthy cohort of dogs.....	24
5.2.2. Changes of PLT, PCT and MPV during treatment.....	27
5.2.3. Attempt to determine a predictive PLT, PCT or MPV value .....	27
6. Discussion.....	29

7. Conclusion.....	31
8. Summary.....	32
10. Bibliography .....	34
11. Acknowledgements .....	37

## **List of abbreviations**

- CD** – Cluster of Differentiation
- DLBCL** – Diffuse large B-cell lymphoma
- MTD** – Maximum tolerated dose
- PLT** - Platelet
- PCT** - Plateletcrit
- MPV** – Mean Platelet Volume
- ROC** – Receiver operating Characteristic
- FACS** – Fluorescence-activated cell sorting
- FNAB** – Fine Needle Aspiration Biopsy
- MST** – Median survival time
- PLTB** – Platelet Base
- PLTV** - Platelet Vincristine
- PLTD** – Platelet Doxorubicin
- PCTB** – Plateletcrit Base
- PCTV** – Plateletcrit Vincristine
- PCTD** – Plateletcrit Doxorubicin
- MPVB** – Mean Platelet Volume Base
- MPVV** – Mean Platelet Volume Vincristine
- MPVD** – Mean Platelet Volume Doxorubicin
- AUC** - Area Under the curve
- OST** – Overall survival time

# 1. Introduction

As many as 20-100 per 100,000 dogs are diagnosed with lymphoma. Canine lymphoma can be comparable with non-Hodgkins lymphoma in humans (Zandvliet, 2016).

Lymphoma is a group of neoplasms of the hemolymphatic system, it accounts for approximately 80% of all hematopoietic tumours. It is a neoplastic proliferation of lymphoid cells with the potential of infiltration into the bone marrow (Meuten, 2017) .

Canine lymphoma can affect any dog, no matter what breed, age, or size but middle-sized to large dog breeds are overrepresented. The incidence rate increases with age, most commonly it is seen in middle-aged to older dogs. (Zandvliet, 2016).

The etiological reason for canine lymphoma is largely unknown and likely multifactorial. Certain genetic and molecular factors have shown to play a role in development of canine lymphoma. Also, immune system alterations such as immune mediated thrombocytopenia has shown a correlation with a higher risk of developing lymphoma compared to a healthy population of dogs (Withrow & MacEwen's Small animal clinical oncology, 2020).

Risk factors such as specific lawn care products showed an increased risk of development of canine lymphoma. Professionally applied pesticides and self-applied insect growth regulators increased the risk of canine lymphoma (Takashima-Uebelhoer, et al., 2012).

Prognostic biochemical markers, molecular genetic markers and immunohistochemistry characters have been identified in aid to diagnose and treat lymphoma. But with more advanced technology also comes higher costs and time-consuming laboratory tasks and skill. It would therefore be beneficial to identify more simple, cheaper, and easily available prognostic markers, that can aid in diagnosing and in prognosis of Canine lymphoma (Li, et al., 2019)

In this retrospective analysis we will investigate the platelets indices of dogs diagnosed with lymphoma to determine if they hold a role in the prognosis of the patients.

## 2. Literature review

### 2.1. An overview of canine lymphoma

Lymphoma develops after the malignant transformation of B- or T lymphocytes and the most common location is the lymph nodes.

Primary lymphatic organs are the bone marrow and the thymus. Extranodal lymphoma is proliferating in other organs than the peripheral lymph nodes, mediastinum, or gastrointestinal tract. Examples of extranodal lymphoma are nasal, respiratory, cutaneous, nervous, and ocular lymphoma. Lymphoma can be characterized by the anatomical location in the patient's body, histological distribution, morphology of the cells, immunophenotype, cytopenia and biological behavior (Meuten, 2017).

Lymphoma cannot be defined as a single disease. The classification system of lymphoma is important for clinical studies performed, to correlate to the difference in biological behavior, the responsiveness to treatment and the prognosis of the different types of lymphoma (Vail, Thamm, & Liptak, *Small Animal Clinical Oncology*, 2019).

#### 2.1.1. Clinical signs of canine lymphoma

Canine lymphoma is diverse in character, and its variable clinical signs are reflected by the affected organs. The different subtypes respond differently to therapy, and the survival rate differs between the subtypes.

When characterized by the anatomical location in the patient's body, the most common forms are multicentric, gastrointestinal, mediastinal, and cutaneous lymphoma, the multicentric form of lymphoma affects the peripheral lymph nodes, it is the most common clinically presented type and more than 80% of dogs with lymphoma are presented with this type.

The gastrointestinal lymphoma is not as common, it represents 5% to 8% of all canine lymphomas. Around 5% accounts for the mediastinal form of the disease. (Zandvliet, 2016).

Multicentric lymphoma presents clinically as enlarged and painless peripheral lymph nodes, with a rubbery texture of the lymph nodes. Mostly the dog does not present any severe signs

of systemic illness, but nonspecific signs such as weight loss, vomiting, fever, diarrhea, ascites, dyspnea, fever, polydipsia, polyuria, and emaciation can occur.

The gastrointestinal form shows symptoms that are nonspecific and seen in many other gastrointestinal tract diseases such as vomiting, diarrhea, weight loss and malabsorption.

Enlargement of the cranial mediastinal lymph nodes and/or the thymus is characteristic for the mediastinal form of lymphoma. Depending on the extent of the disease, clinical signs such as respiratory difficulty, exercise intolerance, regurgitation can be seen, polydipsia and polyuria are also seen occasionally.

Extranodal clinical signs depend on the affected organ, CNS lymphoma show symptoms such as seizures, paralysis, and paresis, while ocular lymphoma is characterized by thickening of the iris, uveitis, glaucoma etc.

Cutaneous lymphomas usually present as nodules, plaques, ulcers, dermatitis with hypopigmentation and alopecia. It can be both mucocutaneous and cutaneous together or separately seen (Vail, Thamm, & Liptak, Small Animal Clinical Oncology, 2019).

### 2.1.2. Diagnosing canine lymphoma

After a thorough physical clinical examination, the quickest and easiest way to diagnose lymphoma is with a fine-needle aspirate. It is the first diagnostic tool of choice. It is sensitive for diagnosing high-grade canine lymphoma, and not considered to be very invasive, it can generally be done on awake patient (Vail, Thamm, & Liptak, Small Animal Clinical Oncology, 2019).

Histopathological analysis is necessary to be able to classify the tumor according to the WHO classification criteria. This is mostly achieved by excising the prescapular lymph nodes and with the aid of immunostains highlighting the architecture of the nodes and the areas affected by the neoplastic lymphocytes whose immunophenotype is determined by the immunostaining.

Additionally, a complete blood count, serum biochemical profile and urine analysis should be done. Hematology analysis is especially needed to confirm leukemia (Richter syndrome)/stage V lymphoma and to screen comorbidities which may alter the treatment course. Most commonly normochromic and normocytic anemia is seen on the blood

hematology and blood smear, hemorrhagic and hemolytic anemia may also be seen. In 30-50% of the cases thrombocytopenia can be seen, but bleeding is not a common clinical problem as is expected (thrombocyte numbers under  $50 \times 10^9/L$  are expected to cause petechiae in dogs (Lilliehöök & Tvedten, 2021)). Lymphocytosis can be seen in affected dog, as well as neutrophilia. In dogs with gastrointestinal lymphoma hypoproteinemia can be observed. Hypercalcemia is also often seen in dogs with lymphoma especially in the T-cell immunophenotype cases. (Vail, Thamm, & Liptak, 2019).

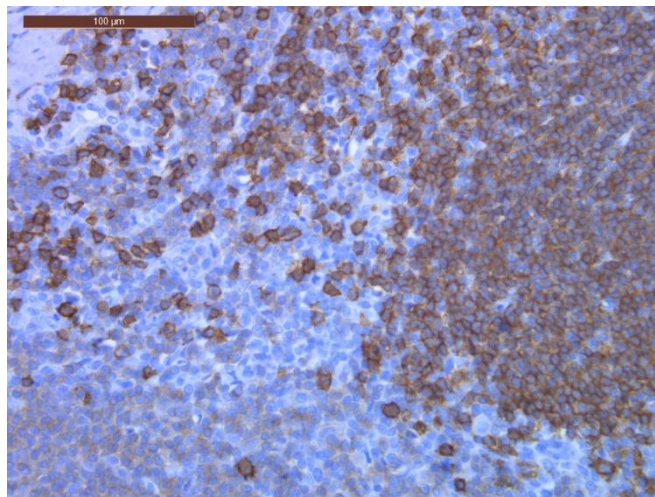
Diagnostic imaging such as ultrasonography and radiography are helpful in staging and seeing the severity and spread of the lymphoma, but not for specific diagnostics. A thoracic radiograph of a canine lymphoma patient will show abnormal findings in 70% of the cases, but these abnormalities are nonspecific, and further diagnostics are necessary. In gastrointestinal lymphoma an abdominal ultrasonography can help in evaluating the extent of the disease and the severity and spread, but further diagnostics are necessary to be sure of canine lymphoma, since many other GI tract diseases can show similar changes (Zandvliet, 2016). Fine needle aspirate is necessary to confirm the presence of the tumor in various thoracic or abdominal organs (e.g., lymph nodes, liver, spleen etc.) are usually obtained with ultrasound guidance.

The staging necessary to describe the extent of the disease, it's made from the result of the physical examination, laboratory tests, diagnostic imaging, and the cytology results. The clinical staging system generally used is the World Health Organization clinical staging system for canine lymphoma. It stages the disease from I-V, with stage five being the most severe case. Stage one is when the lymphoma can only be found in one single node, in stage II multiple nodes are affected in the same area or on the same side of the diaphragm. When the lymphoma is in stage III generalized lymphadenomegaly can be seen, in stage IV there is additional involvement of the spleen and/or the liver. Blood and bone marrow involvement can be seen in stage V. The staging system also has substages a and b, where substage a is without systemic illness, and substage b is with systemic illness, systemic means that the entire body is affected and can give symptoms such as weight loss, fever, seizures etc., rather than a single organ or body part being affected as in substage a. (Ettinger, 2003).

Flow cytometry can be used alternatively to immunohistochemistry to determine immunophenotype in lymphoma. Knowing the immunophenotype will provide additional information for the prognosis and treatment of the patient. To immunophenotype a cell



population, we need to identify their surface antigens or cytoplasm antigens. The cluster of differentiation (CD) is used to identify and investigate leukocytes and other cells connected to the immune system. Monoclonal antibodies that bind to epitopes on specific antigens on the cell, are identified and named CD (cluster of differentiation) followed by a number e.g., CD21, this antigen antibody cluster is found on large diffuse B-cell lymphoma. To be able to see these connections made between the monoclonal antibodies and antigens, the antibodies are labelled with a fluorochrome. Although immunophenotyping will provide additional information that aid in the prognosis and treatment, it cannot replace standard methods of diagnosing and investigating the disease, cytology and histopathology is essential in diagnosing and grading lymphoma (Ilchyshyn, 2016).



*Figure 1 Histopathological image labelled with CD20 for detection of B-cells, courtesy of Valéria Dékay DVM, Mátrix Histopathology and Cytology Ltd, Budapest*

### 2.1.3. Treatment and prognosis of canine lymphoma

Treatment of lymphoma is aimed at controlling the disease, optimally to achieve a complete remission and to provide a good quality of life (Vail, et al., 2009).

The most successful treatment for canine lymphoma is a multiagent systemic chemotherapy protocol, referred to as CHOP (cyclophosphamide, doxorubicin (hydroxydaunorubicin), vincristine (Oncovin) and prednisolone). Lymphoma is sensitive to radiotherapy, but it is mostly only used to reduce clinical symptoms from tumors formed, due to lymphoma being a systemic disease it would be difficult to treat it independently with radiotherapy. The CHOP-protocol therapy generally shows great improvement of the patient with remission seen in 60-90% of the patients, and it is usually a well-tolerated treatment. Relapse is

unfortunately always present within 6-12 months, the aim of the treatment is to prolong life and to give a quality of life (Ettinger, 2003).

CHOP protocol is an injection protocol, that consists of two phases, called induction phase and maintenance phase. The first phase, induction phase, is a more intensive protocol to induce remission in the patient, followed by a short maintenance protocol. The whole treatment is generally over 6 months, but protocols over 12-15 weeks have been shown to be as effective (Zandvliet, 2016).

Cyclophosphamide is an oxazaphosphorine prodrug, in the liver the prodrug is activated via the cytochrome P450 system and forms 4-hydroxycyclophosphamide, which is broken down spontaneously to acrolein and phosphoramidate mustard. Phosphoramidate mustard is the compound that reacts with the DNA and exerts the cytotoxic effect. It is a common chemotherapeutic drug used in veterinary medicine when dealing with oncology. It is often given together with other chemotherapeutic drugs in multidrug protocols. It can be given intravenously or per orally. Cyclophosphamide also has an immunosuppressive effect, and bone marrow suppression is one of the more common toxic effects. Due to the bone marrow suppression the dog can develop thrombocytopenia, leukopenia, neutropenia, and anemia, therefore the patient should be monitored regularly for this. In high doses cyclophosphamide can be cardiotoxic (Warry, Hansen, Gustafson, & Lana, 2011).

Doxorubicin (hydroxydaunorubicin) belongs to the group anthracyclines and is a cytotoxic antibiotic that have been isolated from *Streptomyces peucetius* as early as year 1950, now a days it is produced semisynthetic from daunorubicin. Doxorubicin is a highly tissue irritant drug and extra vasal injection can cause local necrosis. The biological activity in doxorubicin is its capacity to bind to DNA, this inhibits the enzyme systems necessary for DNA replication and transcription. Doxorubicin is most often used in multidrug protocols together with other chemotherapeutic drugs (Rivankar, 2014).

Main side effects of Doxorubicin are bone marrow suppression and cardiac tissue injury. Mainly the neutrophils and platelets are affected by the bone marrow suppression, leading to neutropenia and thrombocytopenia. Cumulative doses of Doxorubicin increase the risk of thrombocytopenia and cardiotoxicity in the patient. The cardiotoxic effect can be acute or chronic. These side effects may have a dose-limiting effect and is a major concern in therapy (Rivankar, 2014).

Vincristine (Oncovin) is an alkaloid isolated from the plant *Catharanthus roseus* and introduced year 1963. Vincristine is an oxidized form from another alkaloid isolated from the same plant, Vinblastine. The pharmacodynamics of Vincristine is not fully known, but its activity is cycle specific and stops the cell division in metaphase by disrupting microtubules when binding to tubulin. (van der Heijden, Jacobs, Snoeijer, Hallard, & Verpoorte, 2004).

Other than treating lymphomas, Vincristine can be used to help treating immune mediated thrombocytopenia. Vincristine has shown to increase platelet count in thrombocytopenic patients, together with glucocorticoids the increase of platelets can be achieved faster than with glucocorticoids alone. An increase in platelet count in dogs with already normal platelet values are also seen after Vincristine administration (Campbell, MacDonald, Dickinson, & Gagnon, 2019).

In Immune mediated thrombocytopenia premature platelets are destroyed by macrophages due to abnormal antibodies bound to the surface of the platelets. Vincristine has been seen to decrease the phagocytosis by the macrophages by its function to impair microtubule assembly. Vincristine has also been suggested to stimulate thrombopoiesis following a short period of impaired megakaryocyte production (Lewis & Meyers, 1996).

The platelet increase causes by Vincristine is not fully known, suggestions are that thrombopoiesis is accelerated, and by impairing phagocytic function of opsonized platelets, less platelets will be phagocytized (Balog, et al., 2013).

The corticosteroid prednisolone is an immunosuppressive steroid. It is often used in treating lymphoma, it is a common and well tolerated drug when used over shorter periods of time. Long term treatment, high doses, or incorrect tapering schedule can cause iatrogenic hyperadrenocorticism.

Prednisolones cause of action is assumed to be the binding to the glucocorticoid receptor 328, which will induce apoptosis in the hematopoietic cancer cells. Prednisolone can also help with managing the side effects and hypersensitivities cause by chemotherapeutic agents (Vail, Thamm, & Liptak, 2020).

Corticosteroids are the cheapest drug of choice in treating canine lymphoma. Using corticosteroid as a single drug treatment in canine lymphoma will in most cases cause drug resistant cancer cells (Hanot, et al., 2018).

Many veterinarian CHOP protocols include L-asparaginase in the treatment against lymphoma. L-asparaginase is an enzyme that converts L-asparagine into aspartic acid and ammonia. L-asparaginase anti-carcinogenic effect against lymphoproliferative disorders has been studied and evaluated for decades, it is used against diseases such as acute lymphoblastic leukemia and Hodgkin's lymphoma. Microbes such as bacteria, microalgae and fungi are the best sources to isolate and purify L-asparaginase from. In treatment of lymphoma and leukemia purified L-asparaginase extracted from *E. coli* and *Erwinia carotovora* microbes are used, due to their higher concentration yield of the enzyme. Two isoforms of L-asparaginase exist when purified from *E. coli*, L-asparaginase I and -II. Isoform II shows more activity against lymphoma (Muneer, et al., 2020).

Lymphoid tumour cells are rapid growing cells which requires large amounts of extracellular asparagine for growth and proliferation. Cells that can synthesize asparagine intracellularly and does not require the high amount for growth are spared when administering L-asparaginase, lymphocytes who lacks the asparagine synthetase will run out of asparagine, this interferes with the tumours DNA and RNA, which leads to a decrease in protein synthesis, and apoptosis of the cells will happen, leading to tumour cell death (McDonald, Thamm, Kurzman, Turek, & Vail, 2005). L-asparaginase leads to lower asparagine levels in the circulation, not only does this affect the tumour negatively, but shown in human studies, also leads to decreased synthesis of important fibrinolytic proteins, procoagulants and anticoagulants. The lack of these factors will result in hypercoagulability and increase the risk of venous thromboembolism (De Stefano, Za, Ciminello, Betti, & Rossi, 2014).

The drugs mentioned above are not always used together in one protocol. The protocols and drugs used may vary depending on the treating veterinarian, the patient, and the owner.

## 2.2. An overview of platelets

Platelets, also called thrombocytes, derive from megakaryocyte cells in the bone marrow, or more correctly, they are fragments of the megakaryocyte cells. One megakaryocyte can produce several thousand platelets. Thrombopoietin is the main cytokine that regulates the megakaryocyte and platelet production, but other cytokines and growth factors also play a role in the regulation. Platelets are disc shaped and the smallest and second most numerous cells circulating in the blood. In their center is the granulomere part with granules containing coagulation factors, along with other cell organelles such as mitochondria, platelets have no

nucleus. Platelets are the most important cells in hemostasis, but also play a major factor in thrombosis, inflammation, and neoplasia. Platelets circulate in the blood for 7-9 days, when they age, macrophages in the spleen and liver remove them from circulation. The number of platelets remain constant in a healthy animal (Weiss & Wardrop, 2010).

### 2.2.1. Platelets significance in disease in general

Platelet pathophysiology in thrombosis and their role in hemostasis is well known, but platelets has shown to have a much more diverse role in the body.

Activated platelets release multiple substances from their different storage granules and lysosomes. These substances have different signaling properties and can therefor influence many different physiological and pathophysiological processes such as inflammation, immunity, angiogenesis, and tumor growth being some examples (Van der Meijden & Heemskerk, 2019).

Platelets function in inflammation is diverse. Platelets have the ability to recognize and kill pathogens, affect the leukocytes by enhancing their ability to phagocytose and kill pathogens, and by activating and recruit leukocytes to the site of inflammation (Jenne & Kubes, 2015).

### 2.2.2. Platelets and cancer

Thrombocytopenia is a common finding in dogs with cancer. In hematopoietic and vascular cancer such as lymphoma it is especially common. There are various reasons for the thrombocytopenia, such as decreased production of platelets, increased removal by the macrophages, destruction, or increased consumption of the platelets (Vail, Thamm, & Liptak, 2020).

There is a high malignant thrombotic risk in cancer patient, this is due to cancer cells being able to activate platelets and stimulate platelet aggregation, this is done through direct and indirect mechanisms. Tumor cell induced platelet aggregation increases the metastatic potential of the tumor. Cancer cells secretion of thrombin is one major factor in platelet aggregation, thrombin converts fibrinogen to fibrin and activates coagulation factors V, VIII,

XI and XIII and the PAR receptors on the platelets which induces the platelet aggregation. Another important activator of the coagulation cascade is when factor VII comes in contact with the tissue factor. Cancer cells and cancer derived particles often express the tissue factor.

By surrounding the tumor cells, platelets also protect the metastatic thrombus from natural killer cells.

Tumor cells express Adenosine diphosphate and acting on cell surface receptors on the platelets it will result in activation of the platelet, the platelet will in response release granule contents, which will activate other nearby platelets.

The cancer cells benefit from this platelet activation in countless of ways, the platelet activation will stimulate tumor growth and it will aid the tumor in metastasis. The release of the granule content releases a big number of growth factors that the tumors thrive from. The growth factors will enhance angiogenesis and tumoral neovascularization (Palacios-Acedo, et al., 2019).

### 2.2.3. Lymphomas effect on platelets

Thrombocytopenia is seen more often in hematopoietic and vascular cancers. Factors contributing to thrombocytopenia in lymphoma can be such as splenomegaly caused by splenic lymphoma, the spleen normally stores one third of the body's platelets. Thrombocytopenia can also be caused by hypercoagulability of the platelets, lymphomatous bone marrow infiltration, and immune-mediated thrombocytopenia (Vail, Thamm, & Liptak, 2020).

Immune-mediated thrombocytopenia such as Thrombocytopenia purpura is a well-known complication of lymphoproliferative disorders. Immune thrombocytopenic purpura is an autoimmune disorder that shows decreased platelet count and mucocutaneous bleeding. It can be primary (idiopathic) or secondary acquired thrombocytopenia from underlying disease such as lymphoma (Liebman, 2007).

There are studies that has proved that platelet indices can be useful as prognostic factors in different types of lymphomas in humans.

Human patients diagnosed with Diffuse Large B-cell lymphoma, and who had low thrombocyte count, based on the Eastern Cooperative Oncology Group scale showed more symptoms such as fever, night sweating and more than 10% body weight loss. Patients with thrombocytopenia also presented more bone marrow involvement and higher International Prognostic Index (IPI is a prognostic factor model for aggressive lymphoma). Thrombocytopenia can be used as an independently poor prognostic factor of overall survival and progression free survival. Platelet count has been reported previously to be useful as a prognostic factor in other haematological malignancies such as splenic marginal zone lymphoma, Richter syndrome, and acute lymphoblastic leukaemia (Ling-Ping, Shyh-Jer, & Ming-Sun, 2012).

Neutrophil Lymphocyte ratio and Platelet Lymphocyte ratio was shown to correlate with faster progression in early-stage Classical Hodgkin lymphoma (Reddy, et al., 2017).

### 3. Goals and hypothesis

The aim with this study is

- To characterize the patient population suffering from high grade B-cell lymphoma treated with MTD chemotherapy.
- To investigate various platelet indices (namely platelet count, mean platelet volume and plateletcrit) in dogs suffering from high-grade multicentric B-cell lymphoma, at 3 different time points: at admission, a week after the first chemotherapy treatment (vincristine in these cases) and following combination treatment (after one vincristine and one doxorubicin treatment was given).
- To compare these indices to a healthy cohort of patients.
- And to evaluate their prognostic significance in this patient population.

Based on previous studies conducted in humans we hypothesize that thrombocytopenia is a negative prognostic indicator in dogs suffering from high grade multicentric B-cell lymphoma.

## 4. Materials and methods

### 4.1. Patients

A retrospective analysis was carried out from medical records of 154 dogs diagnosed and treated for lymphoma at the Veterinary Haematology and Oncology Centre in Budapest. Patients were enrolled if they were diagnosed between January 2014 and November 2020. Survival data collection was closed on September 2021.

#### 4.1.1. Patient selection

The patients were previewed for eligibility. Patients were included in the study if the diagnosis of lymphoma was obtained by fine needle aspiration or histopathology and there was an appropriate staging of the lymphoma and a definite immunophenotype was received either by immunohistochemistry or flow cytometry. Other prerequisites were that the dog has been treated for lymphoma with MTD Chemotherapy, survival and progression data were recorded, and baseline platelet count were available. Patients with no initial thrombocyte count or inconclusive thrombocyte count (analysers marked the thrombocyte result as invalid or platelet clumps were found), patients with indolent lymphomas (marginal zone lymphoma, T-zone lymphoma, small lymphocytic lymphoma, follicular lymphoma) or missing survival data were excluded.

#### 4.1.2. Patient characteristics

The breed of the dogs, their gender status and age were recorded. Data collected was diagnostic methods, histopathology results if available, the extent of the disease, comorbidities, the treatment method used, and the patient's platelet count, plateletcrit and mean platelet volume.

The patient's survival data was collected using the clinic's database as well as the Hungarian microchip registry PetVetData and by phone contact to the dog's owner.



### 4.1.3. Diagnostic methods

The diagnosis of lymphoma was based on appropriate clinical signs and cytology or histopathology. Immunophenotype was determined based on flowcytometry from the lesions, or immunohistochemistry. Immunophenotype was determined based on CD3, CD5, CD20 or CD21 or CD79 immunoreactivity CD3 and CD20 used for immunohistochemistry, all except CD20 for flow cytometry, other antigens were used when necessary (CD45 or CD34). Whenever it was possible, histopathology diagnosis was pursued. Histopathology diagnosis was performed at the University of Veterinary Medicine Budapest or at Mátrix Histopathology Services, the histopathology diagnosis was based on the previously described criteria ( Meuten D. , 2017). Immunohistochemistry was performed at the same sites as histopathology. The staging of the lymphoma was based on World Health Organization clinical staging system for canine lymphoma.

Cytology was performed by Péter Vajdovich DVM dipl ECVCP on slides stained with Reag-Quick (Reagens Kft, Budapest, Hungary)

The staging was performed to meet the WHO staging criteria by assessment of the peripheral lymph nodes, abdominal US and fine needle aspiration biopsy of the spleen/liver/other organs if morphological alterations observed, thoracic imaging by X-ray, complete blood count and basic biochemical analysis including tCa and iCa was performed whenever appropriate. Bone marrow sampling was not performed in every patient only in the patients diagnosed with stage V.

#### 4.1.3.1. Lymph node and bone marrow samples

One of the enlarged prescapular lymph nodes was excised for histological and immunohistochemical examination. The dogs were anesthetized, induction was done with propofol, isoflurane was used for maintenance, continuous rate of fentanyl was set for pain control (propofol: AstraZeneca Co., Cambridge, UK / 5 mg/bwkg iv., isoflurane /Abbott Ltd., Budapest, Hungary / 1.5–2.5 V/V%, fentanyl / Gedeon Richter Nyrt, Budapest, Hungary / by constant rate infusion 0.01 to 0.04 mg/bwkg/h).

With a Jamshidi needle, bone marrow aspirates were taken from the greater tubercle of the humerus. This was done on the dogs that were diagnosed with stage V lymphoma.

Smears were made of the samples for cytological analysis and stained with May-Grünwald and Giemsa (Sigma-Aldrich Co., Saint Louis, Missouri, USA).

#### 4.1.3.2. Histopathology and immunohistochemistry

Histopathology tissue samples were obtained and processed and then stained with haematoxylin and eosin. Each case was serially sectioned and put on positively charged slides for immunohistochemical labelling. CD3 was used for T-cell immunophenotyping, and CD79a or CD20 was used for B-cells. Rabbit anti-CD3 or mouse anti CD79 or CD20 antibody was used. When lymphoma was diagnosed, the WHO classification scheme was used defining the exact diagnosis (i.e., diffuse large B-cell lymphoma, DLBCL). In this study only high-grade B-cell lymphoma was considered.

#### 4.1.3.3. Immunophenotype determination by flow cytometry

Lymph node samples were collected with an aseptic technique using an 18 or 20G needle and 5 ml syringe yielding about 0,5 ml sample. This was stored at 4°C in RPMI medium for a maximum of 48 hrs. Flow cytometric analysis was performed within this timeframe. The cellularity of the sample was checked using a Sysmex xT2000iV haematology analyser. 2 million viable cells were required for a valid analysis (Aniolek, Gajewski, & Gizinski, 2014). Monoclonal antibodies were used for the antigens CD3 (clone: MCA1774, BioRad, Hercules, USA), CD5 (clone: MCA1037PE, BioRad, Hercules, USA), CD21 (clone: MCA1781PE, BioRad, Hercules USA and CD79a (clone: MCA2538PE, BioRad, Hercules, USA). Based on the previously obtained cell count an aliquot containing 2 million viable cells was taken for analysis. Fluorescently labelled antibodies were added to the sample, which was later analysed with a BecktonDickinson FacScan analyser, with FacsComp software.

#### 4.1.4. Treatment methods

Treatment of patients was carried out using MTD chemotherapy protocols depending on the veterinarian's discretion, patient status and owner's request. Intravenous chemotherapy was administered using a closed system drug transfer device (BD PhaSeal).

The described treatments are carried out by the oncology team at the Veterinary Haematology and Oncology Centre in Budapest.

The most common protocol used was the modified CHOP protocol for 20 weeks. Prednisolone (Richter Gedeon Nyrt, Budapest, Hungary) was used in all patients for 4 weeks. A starting dose of 2 mg/kgbw p.o. was given week 1, prednisolone was tapered over 4 weeks. Prednisolone week 2 dose- 1.5mg/kgbw, week 3 dose- 1mg/kgbw, week 4 dose- 0.5 mg/kgbw. Vincristine (Richter Gedeon Nyrt, Budapest, Hungary) was given in a dose of 0.75mg/m<sup>2</sup> i.v. Doxorubicin (TEVA Gyógyszergyár Zrt, Debrecen, Hungary) dose was 30mg/m<sup>2</sup> i.v. Cyclophosphamide (Baxter Co., Deerfield, Illinois) was given in a dose of 200mg/m<sup>2</sup> i.v.

*Table 1 Modified CHOP Protocol 20 Weeks.*

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Vincristine	x		x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	
Doxorubicin		x									x									x
Cyclophosphamide					x			x						x			x			
Prednisolone	x	x	x	x																

#### 4.1.5. Assessment of remission, relapse, and survival data

Remission and relapse were assessed according to VCOG RECIST criteria (Vail, et al., 2009).

In this study responses as well as survival times and time to progression was recorded (for definitions see table 2 and 3).

Table 2 General disease response definitions ( Vail, et al., 2009)

<b>Lesion response</b>	<b>Definition</b>
Complete response (CR)	Target lesions: Disappearance of all evidence of disease. All lymph nodes must be non-pathologic in size in the judgment of the evaluator(s). Non-target lesions: Any pathologic lymph nodes must be considered to have returned to normal size in the judgment of the evaluator(s), and no new sites of disease should be observed. Spleen and liver should be considered within normal limits by the evaluator(s).
Partial response (PR)	Target lesions: At least a 30% decrease in the Mean Sum LD of target lesions taking as reference the baseline mean sum LD. Non-target lesions: Not applicable.
Progressive disease (PD)	Target lesions: At least a 20% increase in the Mean Sum LD taking as reference the smallest mean sum LD at baseline or during follow-up (this includes the baseline mean sum LD if that is the smallest on study). The LD of at least one of the target lesions must demonstrate an absolute increase of at least 5 mm compared with its nadir for PD to be defined. For target lesions
Stabile disease (SD)	Target lesions: Neither sufficient decrease to qualify for PR nor sufficient increase to qualify for PD. Non-target lesions: Not applicable

If the dog relapses after the initial protocol, further treatment options were a second cycle of received 20 weeks cycle of the modified CHOP protocol, or additional Madison-Wisconsin protocol, starting with L-asparaginase injection (medac Gesellschaft für klinische Spezialpräparate Bratislava, Slovakia) once in a dose of 400 IU/kgbw SC. After the second cycle, a 3rd cycle of protocols is given to relapsed dogs. Relapse treatment is dependent on the dog's status and chosen after discussion with the owner.

Table 3 Response duration end point definitions (Vail, et al., 2009)

<b>End point</b>	<b>Patients</b>	<b>Definition</b>	<b>Measure from</b>
Overall survival	All	Death as a result of any cause	Initiation of treatment
Time to progression	All	Time to progression or death from lymphoma	Initiation of treatment

By measuring the size of the prescapular lymph node remission rate was estimated. A calliper is used for the measurement of the lymph node. The lymph nodes size is measured in at least one dimension. Complete remission is recorded if the lymph nodes size has reduced by 100%, normal lymph node size or is hardly palpable.

## 4.2. Reference population

Blood from 43 client-owned dogs were collected at the University of Veterinary Medicine Budapest as part of a general health check requested by the owner. A physical examination as well as a complete blood count and basic biochemical tests were performed to assess their health. Only 31 of these dogs were used for this study. The others were omitted due to various alterations observed during the physical examination, haematology analysis or biochemical tests indicating problems which may alter platelet counts.

## 4.3. Blood samples and platelet analyses

Blood was withdrawn from the patients cephalic or jugular vein with a single venipuncture in a closed system and submitted for analysis within an hour. The thrombocyte levels and indices were measured by Sysmex XT2000iV analyzer (Sysmex Hungary Kft., Budapest) and an Abacus Junior Vet 5 analyzer (Reagens Kft, Budapest. Hungary). The latter was only used for some of the lymphoma bearing patients.

Platelet measurements were checked for validity especially if low platelet numbers were observed. Haematology reports were assessed whether the analyser gave any indication of an invalid measurement as well as PLT histograms. Manual PLT counts were also checked to see whether they correlated with the automated ones. In case the automated cell count was inadequate the result was omitted.

PLT, PCT and MPV were recorded from the reference population as well as from the patient population. The latter included 3 different timepoints: at admission, a week after the first vincristine treatment and a week after the first doxorubicin treatment for assessment of changes and comparison.

## 4.4. Statistical analyses

Statistical analyses were carried out using the program R version 4.1.1 (Foundation for Statistical Computing, Vienna) with survival and receiver operating characteristic (ROC) packages.

Kaplan-Meier method and Logrank test was used in time to event analysis.

Shapiro-Wilk test was used to test for normality of platelet related data. Friedman rank-sum test was used to compare reference and patient data. Treatment associated changes in platelet indices were assessed using a paired Wilcoxon test. PLT, PCT and MPV were investigated for their predictive value for 3, 6, 12 and 24 months (used as stratifiers, see below) as well as for the median survival time. The patients were stratified using these cut-offs to create two populations, controls and cases (i.e., those reaching the cut-off and those who didn't reach it). Only patients who died due to lymphoma were used. PLT, PCT and MPV values were assessed for their sensitivity and specificity. ROC curves were plotted. The given biomarker was considered useful if the area under the curve exceeded 0,7. In this case a cut-off point was selected, and sensitivity and specificity recorded too.

## 5. Results

### 5.1. Eligible patients

67 of the 154 patients found with the keyword 'lymphoma' met the inclusion criteria. Of the initial 154 patients 11 had inadequate follow-up data, 35 inadequate immunophenotyping, 23 presented with T immunophenotype, 13 presented with an indolent disease or extranodal involvement. In 53 cases treatment protocols did not meet the inclusion criteria (metronomic treatment or untreated cases); pre-treatment lab results were incomplete in 3 cases. Cases were omitted based on one or more of these.

### 5.1.1. Patient characteristics

Gender distribution of the 67 patients was 38 male and 29 females, 26 dogs were neutered, 40 intact and one of the patients is missing this data. The median age of the patients was 7.8 +/- 2.9 years, age range was 3-15 years. Breed distribution is shown in table 4.

*Table 4 Breeds represented by more than one patient.*

<b>Breed</b>	<b>Number of dogs</b>
Mixed	15
Hungarian vizsla	10
French bulldog	4
German shepherd	3
Labrador retriever	3
American Staffordshire terrier	2
Other	30

### 5.1.2. Tumor characteristics of the patients

In 31 patients Histopathology was performed (29 of these dogs had DLBCL and 2 had lymphoblastic lymphoma), Fine needle aspiration biopsy (FNAB) and flow cytometry performed in 37 cases. Histopathology and flow cytometry was performed simultaneously in 17 of these patients.

Only patients with stage III, IV and V were represented in this study, the stage distribution is seen in table 5.

*Table 5 Stage distribution of the patients*

<b>Stage</b>	<b>Number of patients</b>
III	12
IV	41
V	14

Substage A was seen in 49 patients and Substage B in 18 patients.

Comorbidities was found in two patients, one patient had mammary carcinoma, and the other patient had degenerative mitral valve disease.

### 5.1.3. Treatment and survival of the patients

Prednisolone was used in all patients in a dose of 2 mg/kgbw and tapered in the first 4 weeks as part of the treatment protocol. MTD protocols followed are outlined in table 6, Vincristine based protocols indicate patients who didn't receive other medication then vincristine and prednisolone.

Table 6 Treatment distribution among patients.

<b>Treatment</b>	<b>Number of patients</b>
CHOP 20 week	55
Vincristine based	6
Madison-Wisconsin 25 week	3
Doxorubicin + metronomic cyclophosphamide	2
Madison-Wisconsin 25 week without Asparaginase	1

Recorded treatment responses are given in table 7.

Table 7 Remission of the patients.

<b>Lesion response</b>	<b>Number of patients</b>
CR	57
PR	3
SD	1
PD	1
NA (Not Applicable)	5

Survival analysis: 44 patients died due to lymphoma, 12 patients were lost to follow up and 10 patients died due to other cause. One patient was still alive when survival data collection was stopped. Patients who were lost to follow up or died due to other reasons were censored. The overall survival of high grade multicentric B cell lymphomas: Median survival time (MST) was 328 days (95 % CI 289-541 days), 32,8% survived 1 year, 14,9% survived for 2 years. The median time to progression was 266 days (figure 2). Survival advantage was examined in this group of patients: stage (figure 3, table 8), substage (figure 4, table 9) and age (figure 5, table 10) appeared to carry survival advantage in this patient cohort. Gender, neuter status, and corticosteroid pre-treatment was also examined. No significant differences were found for these parameters.



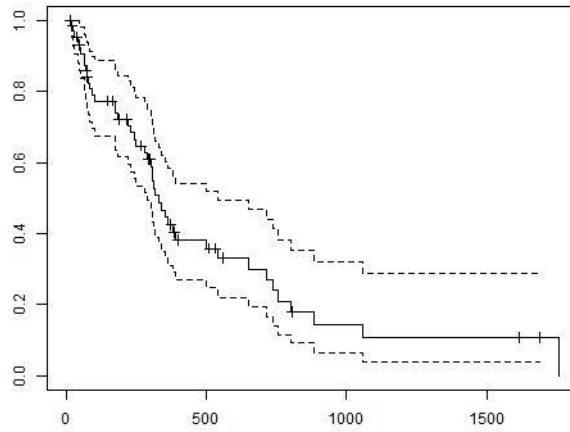


Figure 2 Kaplan-Meier survival curve of patients suffering from multicentric high grade lymphoma treated with MTD chemotherapy. x axis represents survival ratio, y axis the elapsed time in days. Censored cases are indicated with crosslines. Dashed lines indicate confidence interval.

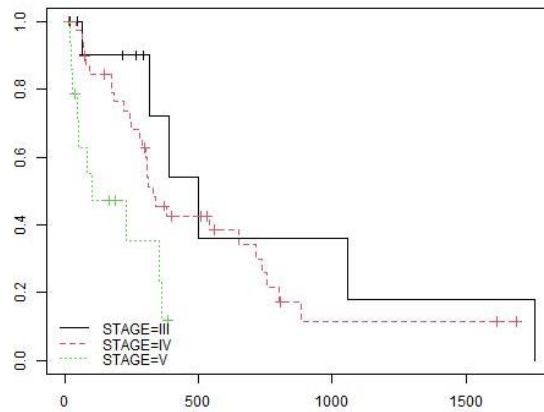


Figure 3 Kaplan-Meier survival curve of patients suffering from multicentric high grade lymphoma treated with MTD chemotherapy. x axis represents survival ratio, y axis the elapsed time in days. Censored cases are indicated with crosslines. Solid line indicates Stage III, dash lines indicate Stage IV and dotted line indicates Stage V.  $p= 0.008$

Table 8 Survival analysis based on Stages.

Stage	Patients	Deaths	Median survival time days	95% confidence interval
III	12	6	498 days	318-NA
IV	40	28	328 days	289-736
V	14	10	101 days	51-NA

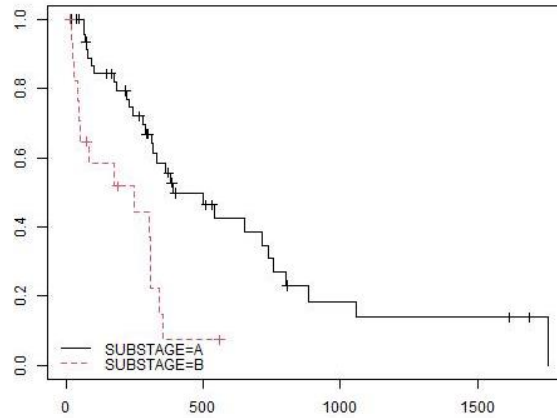


Figure 4 Kaplan-Meier survival curve of patients suffering from multicentric high grade lymphoma treated with MTD chemotherapy. x axis represents survival ratio, y axis the elapsed time in days. Censored cases are indicated with crosslines. Solid line indicates Substage A, dashed line indicates Substage B.

Table 9 Survival analysis based on Substages.

Substage	Patients	Deaths	Median survival time days	95% confidence interval
A	48	30	391	318-757
B	18	14	249	51-351

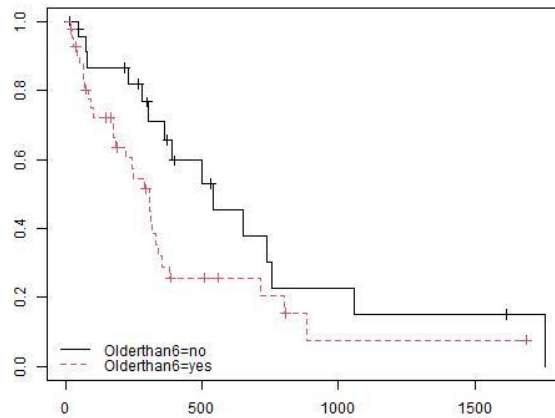


Figure 5 Kaplan-Meier survival curve of patients suffering from multicentric high grade lymphoma treated with MTD chemotherapy. x axis represents survival ratio, y axis the elapsed time in days. Censored cases are indicated with crosslines. Solid line indicates dogs younger than 6 years. Dashed lines indicate dogs older than 6 years.  $p = 0.05$

Table 10 Survival analysis based on age.

Age	Patients	Deaths	Median survival time days	95% Confidence interval
Under 6 years	24	15	541	360-NA
Over 6 years	42	29	305	185-351

Median time to progression was also investigated. Significant differences were found with respect of the stage (figure 6, table 11) and substage (figure 7, table 12) of the disease. Similar parameters as for the survival were examined without finding a significant difference for time to progression.

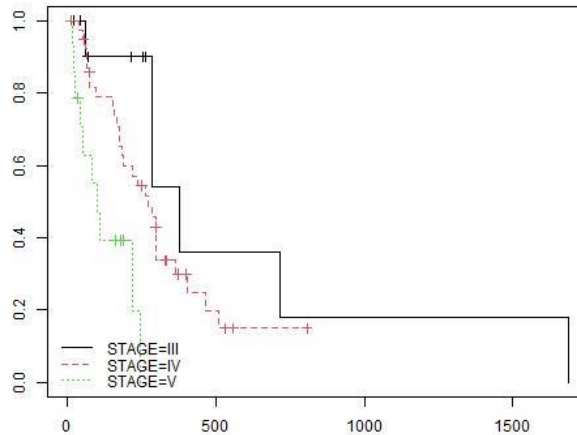


Figure 6 Kaplan-Meier survival curve of patients suffering from multicentric high grade lymphoma treated with MTD chemotherapy. x axis represents survival ratio, y axis the elapsed time in days. Censored cases are indicated with crosslines. Solid line indicates Stage III, dash lines indicate Stage IV and dotted line indicates Stage V.  $p=0.001$

Table 11 Progression analysis based on Stages.

Stage	Patients	Deaths	Median time to progression	95% confidence interval
III	12	6	380	284-NA
IV	40	28	275	185-406
V	14	10	101	51-NA

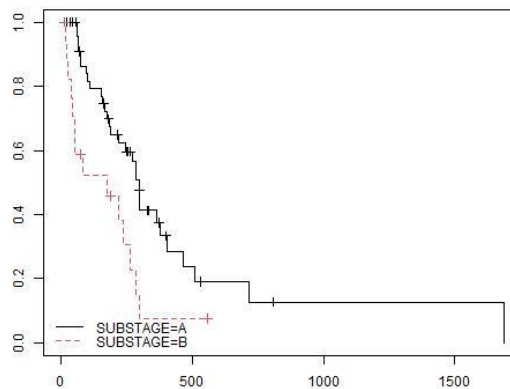


Figure 7 Kaplan-Meier survival curve of patients suffering from multicentric high grade lymphoma treated with MTD chemotherapy. x axis represents survival ratio, y axis the elapsed time in days. Censored cases are indicated with crosslines. Solid line indicates Substage A, dashed line indicates Substage B.  $p=0.006$

Table 12 Progression analysis based on Substages.

Substage	Patient	Deaths	Median time to progression days	95% confidence interval
A	48	30	299	221-464
B	18	14	175	51-301

## 5.2. Platelet indices of the patients

### 5.2.1. Comparison of PLT, PCT and MPV to a healthy cohort of dogs

PLT, PCT and MPV were recorded, the results are indicated in table 15 below.

Table 13 Comparison of lymphoma diagnosed and treated patients to a healthy dog population, values compared were PLT, PCT and MPV.

Analyte	Unit	Number of patients	Mean	Median	Range
PLT Reference population	10 <sup>9</sup> /L	31	243	239	154-388
MPV Reference population	fL	31	9,8	9,3	7,4-13,5
PCT Reference population	%	30	0,23	0,22	0,14-0,35
PLT Baseline	10 <sup>9</sup> /L	67	225	220	15-527
MPV Baseline	fL	47	10,0	10,0	7,6-12,3
PCT Baseline	%	47	0,25	0,23	0,05-0,49
PLT post vincristine	10 <sup>9</sup> /L	54	387	365	93-772
MPV post vincristine	fL	30	9,9	9,9	8,3-12,4
PCT post vincristine	%	30	0,38	0,39	0,10-0,73
PLT post doxorubicin	10 <sup>9</sup> /L	39	267	261	9-600
MPV post doxorubicin	fL	26	9,9	9,9	6,5-12,7
PCT post doxorubicin	%	26	0,27	0,26	0,07-0,48

Friedman rank sum test comparing the reference population to the tumor bearing patients revealed significant differences between the following parameters:

PLT and PLTB (p-value <0.05)

PLT and PLTV (p-value <0.001)

PCT and PCTV (p-value <0.05)

The platelet count from the reference population showed a significant difference from the baseline platelet count of dogs suffering from lymphoma, the platelets count after the vincristine treatment also showed a significant difference.

PCT of the reference population showed a difference than that of the dogs treated with vincristine.

Other parameters tested against each other was PLT vs PLTD, PCT vs PCTB, PCT vs PCTD, MPV vs MPVB, MPVV or MPVD. These results generated no significant difference.

Figures 8, 9 and 10 are showing PLT, PCT and MPV values respectively from the reference population and during treatment.

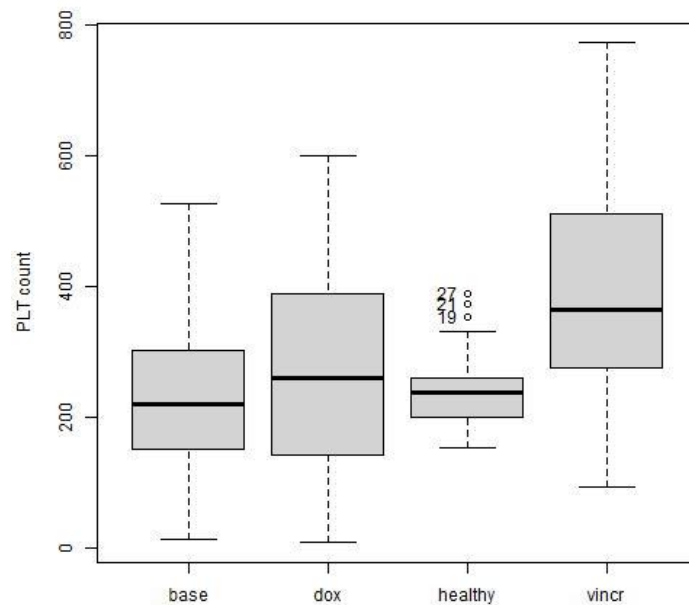


Figure 8 Boxplot of PLT values from a healthy cohort of dogs, and dogs suffering from lymphoma baseline=base, post-vincristine=vincr etc). Outliers are indicated with empty circles.

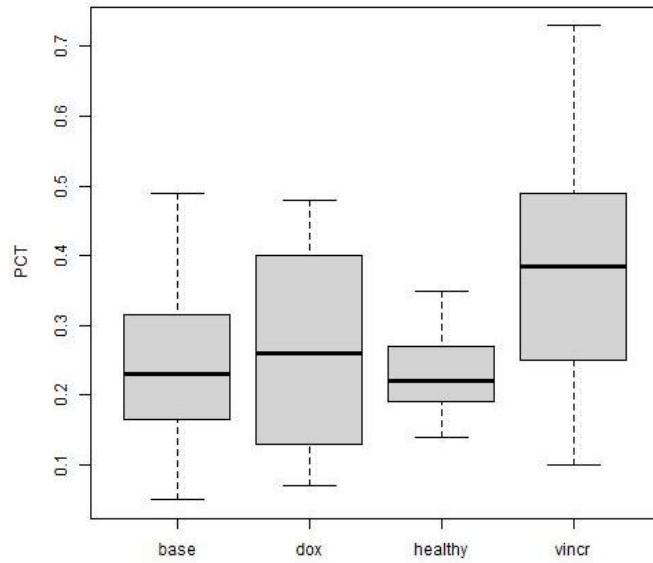


Figure 9 Boxplot of PCT values from a healthy cohort of dogs, and dogs suffering from lymphoma baseline=base, post-vincristine=vincr etc). Outliers are indicated with empty circles.

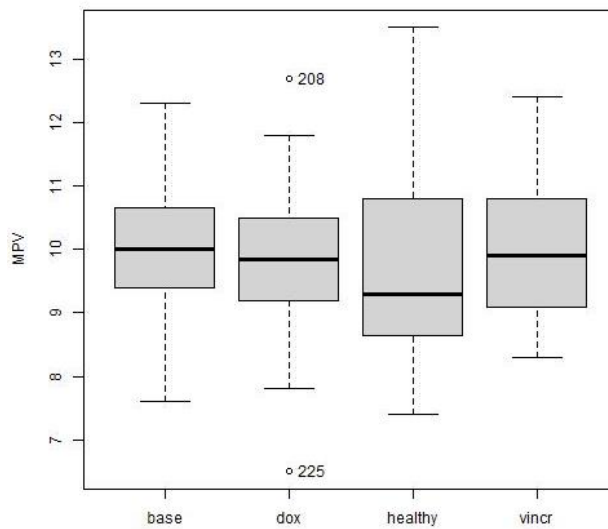


Figure 10 Boxplot of MPV values from a healthy cohort of dogs, and dogs suffering from lymphoma baseline=base, post-vincristine=vincr etc). Outliers are indicated with empty circles.

### 5.2.2. Changes of PLT, PCT and MPV during treatment

Wilcoxon signed rank test revealed the following significant differences:

PLTB and PLTV (p-value < 0.0001)

PCTB and PCTV (p-value < 0.0001)

Other parameters tested were PLTB vs PLTD and PLTV vs PLTD, as well as PCTB vs PCTD and PCTV vs PCTD as well as MPVB vs MPVV and MPVB vs MPVD, and MPVV vs MPVD, but these failed to show significant difference.

### 5.2.3. Attempt to determine a predictive PLT, PCT or MPV value

Different ROC curves were plotted as described previously. From these ROC curves using PCTB as a marker and 3- or 6-months survival as a control/case separator appeared to be promising with area under the curve (AUC) over 0.7 (figure 11 and 12).

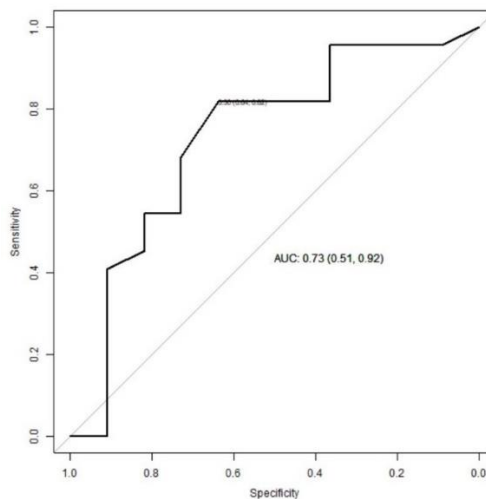


Figure 11 ROC curve for PCT using 3 months survival as patient stratifier. AUC=0,73, PCT cut-off selected at 0,30 % with a sensitivity of x and a specificity of y.

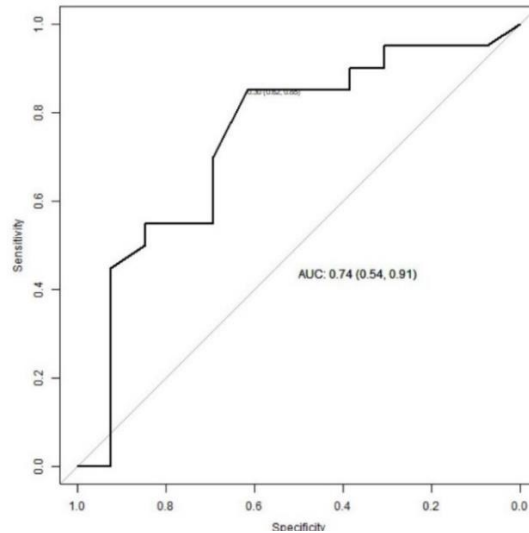


Figure 12 ROC curve for PCT using 6 months survival as patient stratifier. AUC=0,74, PCT cut-off selected at 0,30 % with a sensitivity of x and a specificity of y.

Cut-off value: if the PCT at baseline is more than 0,30 then the survival seems to become worse. The cut-off value was then checked using the data from the 67 patients with the following results (figure 13 and table 14).

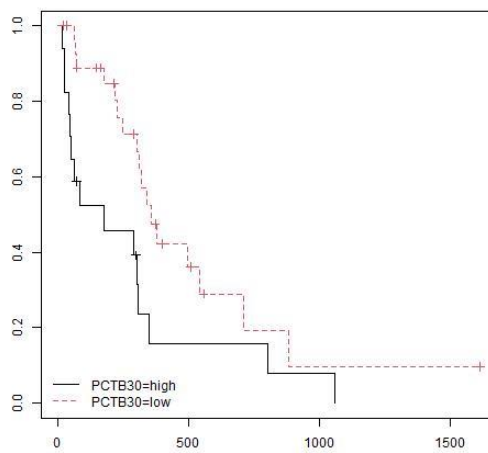


Figure 13 Kaplan-Meier survival curve of patients suffering from multicentric high grade lymphoma treated with MTD chemotherapy. x axis represents survival ratio, y axis the elapsed time in days. Censored cases are indicated with crosslines. Solid line indicates patients with PCTB over 0.3 Dashed lines indicates patients with PCTB under 0.3.  $p=0.02$ .

Table 14 Cut-off analysis based on PCTB.

PCT30B	Patients	Deaths	Median survival time days	95% Confidence interval
High	17	15	175	51-NA
Low	29	17	360	305-NA



## 6. Discussion

Our patient distribution shows that most of the dogs in the current study are mixed breeds or Hungarian vizslas, according to other studies for e.g., Bullmastiff, Rottweiler and Scottish terrier shows more prevalence to be affected by canine lymphoma (Zandvliet, 2016). Mixed breeds represent the majority of the world's dog population and therefore it's no surprise it represents the majority of the dogs in the current study, it has also shown to be one of the more diagnosed group of dogs according to other studies. The Hungarian vizsla being Hungary's national dog, one can assume that this dog is more common in Hungary than others.

The overall survival data of this study showed a median survival time of 328 days, this does not differ much from previous studies done (Dhaliwal, Kitchell, Ehrhart, Valli, & Dervisis, 2013). We see a slightly higher MST in this current study, due to B-cell malignancies being more chemosensitive we can assume it would yield in better results when chemotherapy is attempted (Marconato, et al., 2011).

We can see a 1-year OST in 32,8%, and a 2-year OST in 14,9% of the patients in this study. In a canine lymphoma review written by M. Zandvliet 2016 we can see a 1-year OST of 20-44% with different treatment protocols. A 2-year OST of 0-24% was seen with different treatment protocols (Zandvliet, 2016). The result in this study reflects the survival data collected in this review.

Only patients staged with III, IV and V are represented in the current study, reasons for this could be the small patient population that was eligible for the study, but also lower stages are harder to detect by owners and it could be that the disease has progressed further before owners seek help from veterinarians, this reflects the smaller number of studies of stage I-II disease in international studies.

When age was tested for, dogs aged 6 years or less showed a longer MST. In many studies age has not shown to have a significant impact of survival time in treated dogs. But factors such as increased risk of adverse effects from the chemotherapy, and the older dog's ability to tolerate the adverse effects as well as a younger dog might bear some impact. Older dogs can also be assumed to carry more comorbidities, although this was checked for in this study, sometimes undetected comorbidities might exist.

Median time to progression was also investigated. Significant differences were found with respect of stage of disease. Stage 3- 380 days, stage 4 -275 days, stage 5- 101 days. As expected, a lower stage of the disease results in better prognosis.

The post vincristine values showed increased PLT-count and a higher PCT from both the reference group and baseline values. It is expected that the vincristine will increase the platelet count (Balog, et al., 2013).

There are studies that has proved that platelet indices can be useful as prognostic factors in different types of lymphomas in humans. Patients with thrombocytopenia also presented more bone marrow involvement and higher International Prognostic Index (IPI is a prognostic factor model for aggressive lymphoma). Thrombocytopenia can be used as an independently poor prognostic factor of overall survival and progression free survival. Platelet count has been reported previously to be useful as a prognostic factor in other haematological malignancies such as splenic marginal zone lymphoma, Richter syndrome, and acute lymphoblastic leukaemia (Ling-Ping, Shyh-Jer, & Ming-Sun, 2012)

Other human studies of patients with myelodysplastic syndrome (MDS) showed that severe thrombocytopenia is associated with poor prognosis. Overall survival by platelet response in patients with severe thrombocytopenia, a significant survival advantage was observed in patients who achieved a platelet response (Tang, et al., 2018).

In this study we see that the results of PCTB being more than 0,30 generates a worse survival data, this is against know literature. Our hypothesis was based on previous studies conducted in humans that thrombocytopenia is a negative prognostic indicator in dogs suffering from high grade multicentric B-cell lymphoma.

But platelets also have shown to aid the tumors cells, the cancer cells can activate platelets and benefit from this platelet activation in countless of ways, the platelet activation will stimulate tumor growth and it will aid the tumor in metastasis. The release of the granule content releases a big number of growth factors that the tumors thrive from. The growth factors will enhance angiogenesis and tumoral neovascularization (Palacios-Acedo, et al., 2019)

In this study MPV values were also examined, generating no significant results. In human studies MPV has shown to bear a prognostic value, as an example it has been demonstrated that low baseline MPV can be used as an independent prognostic marker of poor outcome in human patients with DLBCL (Zhou, et al., 2017).

PLT was also investigated but our efforts were not rewarded in this study. PCT appeared to show promise in prognosticating lymphoma and considering that MPV did not appear to be significantly different with treatment and with respect to the reference population it was hoped that PLT would be a good candidate for prognostication. One reason for this finding may be the different number of measurements for the two parameters.

## 7. Conclusion

Based on previous studies conducted in humans we had hypothesized that thrombocytopenia would be a negative prognostic indicator in dogs suffering from high grade multicentric B-cell lymphoma.

The results give us a cut-off value that tells us that if the PCT at baseline is more than 0.30, the survival seems to become worse. This could be a potentially clinically useful marker. The results may not be true since it is against known literature in humans, it is also the opposite of what was expected in the hypothesis. The limitation for this result is that our population is small, a low number of data is tested for, and it is not tested on an independent population. Therefore, testing it on a larger (and different) patient population appears to be a good idea.

Other results in this study:

The overall survival data of this study showed a median survival time of 328 days, this does not differ much from previous studies done. We see a slightly higher MST in this current study, this could be due to B-cell malignancies being more chemosensitive so we can assume it would yield in better results when chemotherapy was attempted.

We can see a 1-year OST in 32,8%, and a 2-year OST in 14,9% of the patients in this study, this seem to be in alignment with other studies performed.

The median time to progression in this study was 266 days, considering our patients group and histopathological diagnoses performed this shows similar results as other studies performed.

As expected, the post vincristine values showed increased PLT-count and a higher PCT from both the reference group and baseline values.

Significant differences were found when investigating the median time to progression with respect of stage of disease. Stage III- 380 days, stage IV -275 days, stage V- 101 days. As expected, a lower stage of the disease results in better prognosis.

In this study MPV values were also examined, generating no significant results. In human studies MPV has shown to bear a prognostic value.

As a final conclusion, this study has many results that are in alignment with previous studies performed. But the shortcomings in this study should not be neglected. The low number of patients, that our patient group does not have a homogenous testing base before diagnosis, that the patients are not all under the same type of treatment protocols may influence results. The low number of data tested for, and that it is not tested on an independent population should also be considered.

## 8. Summary

In this retrospective analysis we investigated the platelets indices of dogs diagnosed with lymphoma to determine if they hold a role in the prognosis of the patients, in hope to find a more simple, cheaper, and easily available prognostic marker, that can aid in the prognosis of Canine lymphoma.

The aim was to characterize various platelet indices in dogs suffering from high-grade multicentric B-cell lymphoma.

Our hypothesis was based on previous studies conducted in humans, that thrombocytopenia is a negative prognostic indicator in dogs suffering from high grade multicentric B-cell lymphoma.

The patients were previewed for eligibility. The breed of the dogs, their gender status and age were recorded. Data collected were diagnostic methods, histopathology results if available, the extent of the disease, comorbidities, what treatment method used, and the patient's platelet count, plateletcrit and mean platelet volume. The diagnosis of lymphoma was based on appropriate clinical signs and cytology or histopathology. Immunophenotype was determined based in flowcytometry from the lesions, or immunohistochemistry. Treatment of patients was carried out using MTD chemotherapy protocols depending on the

veterinarian's discretion, patient status and owner's request. The most common protocol used was the modified CHOP protocol for 20 weeks.

Responses as well as survival times and time to progression was recorded.

A reference population of 31 healthy client-owned dogs was used to compare the values of the patient group in this study.

Our only clinically useful marker obtained was PCT value at baseline, the results showed that a cut-off value at baseline is more than 0.30, the survival becomes worse. This is against known literature in humans, and the opposite of what we expected in the hypothesis. There are shortcomings in this study, and more testing is required on a larger population group and an independent population group

Other results obtained in this study was in alignment with previous performed studies.

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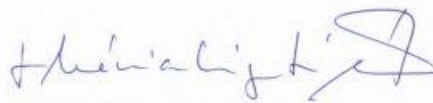
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