European Canine Lymphoma Network
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Fourth Meeting of the European Canine Lymphoma Group, CH-Lugano, June 22nd 2019

How to stage Canine Lymphoma in 2019

Workshop Proceedings

Edited by

Franco Guscetti

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The abstracts of the poster and oral presentations session have been accepted after peer-review by a scientific committee composed by F. Guscetti, L. Marconato, S. Comazzi and L. Aresu under strict avoidance of conflicts of interests.

Welcome from the Organizing Committee

Lugano, June 22nd, 2019

Dear colleagues,

the meeting of the European Canine Lymphoma Network as a satellite workshop to the International Conference on Malignant Lymphoma (ICML) in Lugano has reached its 4th edition. We wish to acknowledge the continued kind invitation of the 15-ICML organizers to join. Indeed, canine lymphoma has continued to arouse interest for its potential as a comparative model. The European Canine Lymphoma Network (www.eu-can-lymph.net), born in 2010 and now linking more than 70 researchers from 25 European institutions involved in research and cure of canine lymphoma, constantly strives towards this goal.

The workshop will start with an invited plenary lecture held by Anne Avery from the University of Colorado. Dr. Avery will give an overview on the variability of outcomes for different types of canine lymphoma. This emphasizes the central importance of an adequate classification for both the clinical management and scientific research on canine lymphoma.

This year's workshop is centered on staging, a topic that we considered worth discussing in the light of its impact on the management and study of canine lymphoma. Review presentations will explore aspects of staging from different perspectives and also highlight shortcomings of the current practice. A round table discussion among experts, open to all the audience, will follow, with the goal to create the basis for a draft document containing guidelines on staging canine lymphoma for the perusal of the members of the ECLN network. Ideally this document could define, for the main lymphoma subtypes, the minimal panel of tests necessary for adequately predicting biological behavior.

As it has become tradition, we will enjoy the coffee break while having the chance to discuss the posters which have been peer-reviewed by the scientific committee and which present interesting novel aspects of canine lymphoma research. This year we accepted a total of 12 abtracts dealing with a disparate number of topics including clinical, diagnostic and more basic research-related subjects. As a novelty compared to the past editions, we have chosen three of the abtracts for oral presentations. This is meant to give greater visibility to interesting approaches.

The high quality of speakers and the large number of pre-registrations makes us confident that the 4th workshop will be as successful as the previous ones and that it will further contribute to strenghten international collaboration. We wish that our initiative will also contribute to foster further interest in this topic in the future.

Enjoy the workshop!

The organizing committee,
Stefano Comazzi, Laura Marconato, Luca Aresu and Franco Guscetti

Programme

13:00 -13:30 Registration

13:30-13:45 **Welcome and update on the European Canine Lymphoma Network activities**
S. Comazzi, L. Marconato - IT

INVITED LECTURE

13:45-14:30 The scientific and clinical utility of disease classification in canine lymphoma
A. Avery, University of Colorado - US

STAGING OF CANINE LYMPHOMA

14:30-15:45 **State of the art of lymphoma staging**

- 14:30-14:45 Clinical staging in canine non-Hodgkin lymphoma
  O. Škor - AT
- 14:45-15:00 Role of Cytology and Flow cytometry in clinical staging
  B. Ruetgen - AT, F. Riondato - IT, S. Comazzi - IT
- 15:00-15:15 Role of Histopathology, Immunohistochemistry and PARR in clinical staging
  F. Guscetti - CH, L. Aresu -IT
- 15:15-15:45 Shortcomings of the current practice of staging: the example of DLBCL vs MZL
  L. Marconato - IT

15:45-16:30 **Round table: how to stage canine lymphoma in 2019?**
A proposal for European Guidelines

16:30 – 17:00 **Poster viewing and coffee-break**

SELECTED ORAL PRESENTATIONS

17:00-17:15 Clinicopathological characteristics and prognostic factors for canine multicentric non-indolent T-cell lymphoma: 107 cases
K Purzycka -UK

17:15-17:30 Are B-symptoms more valuable than substage B to predict prognosis in canine nodal diffuse large B-cell lymphoma?
O. Škor - AT

17:30-17:45 RNA sequencing of canine cutaneous epitheliotropic lymphoma and immune-mediated dermatoses reveals major differences in cell-matrix adhesion and ribosomal proteins
M. Dettwiler - CH

17:45-18:00 **End of works and take home message**

Invited lecture

The scientific and clinical utility of disease classification in canine lymphoma

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Lymphoproliferative disorders are a highly heterogeneous group of diseases that vary in their genetics, epidemiology and outcomes. In people there are more than 50 discreet subtypes of lymphoma and leukemia as classified by the World Health Organization, and in dogs there are at least a dozen well recognized forms of nodal and extra-nodal lymphoma and leukemia. On one end of the canine outcome spectrum are precursor neoplasms (acute leukemia), which have a median survival time that is generally measured in days despite aggressive chemotherapy. At the other end of the spectrum is T zone lymphoma, an indolent disease that often does not require therapy, and which has median survival times of greater than 2 years. This talk will provide an overview of outcomes in many types of lymphoma, with a focus on three forms – precursor lymphoma/leukemia (acute leukemia), peripheral T cell lymphoma and B cell chronic lymphocytic leukemia/small cell lymphoma. These diseases span the spectrum of clinical outcomes, and studies in each of these areas provide a roadmap for expanding our diagnostic capabilities across all disorders.

Precursor lymphoma/leukemias can present in lymph nodes, and be classified as "lymphoma" if cytology and clinical signs alone are used for diagnosis. This disease, however, has a very rapid clinical course and recognition early can help owners and veterinarians make informed decisions. By definition, precursor neoplasms arise from cells that are developing in either the bone marrow or thymus, and therefore presentation as a mediastinal mass, peripheral cytopenias or leukemia is common. Expression of CD34, as detected by flow cytometry, is considered diagnostic for precursor neoplasia, although CD34 expression does not differentiate lymphoid from myeloid leukemia, nor does it differentiate precursor B cell neoplasia from precursor T cell neoplasia. Not all acute leukemias will be CD34 positive, although the majority are. The distinction between AML and ALL is also not always clear by cytology alone. Gene expression profiling studies demonstrate that expression of CD14 can be used to identify AML, and the expression of CD5 identifies T cell ALL. Regardless of lineage, this disease has a very poor prognosis [1-4].

Nodal peripheral T cell lymphoma is the most common form of mature T cell neoplasm in dogs. This group of diseases is highly heterogeneous, both histologically, cytologically and immunophenotypically. The normal counterpart of peripheral T cell lymphomas is probably variable, reflecting the multiple different types of normal T cells which undergo neoplastic transformation. The most common form in dogs is characterized by expression of CD4, low levels of class II MHC, frequent loss of the T cell antigen CD5, frequent hypercalcemia and mediastinal involvement. The immunophenotype determined by flow cytometry reliably predicts the histologic subtype and outcome [5, 6]. Even though mediastinal involvement is common with CD4+ peripheral T cell lymphoma, gene expression profiling demonstrates that these cells do not express TdT, indicating

they are derived from a T cell that has completed development. The phenotype of these cells suggests they may be derived from naïve T cells that have matured in the thymus, but have not yet been activated by antigen. Non-CD4+ nodal T cell lymphomas – those expressing CD8 or neither CD4 nor CD8 – are histologically diverse but have a similar outcome to CD4+ T cell lymphoma (Harris, in preparation). These are also considered to be PTCL until they are further subclassified by gene expression profiling and mutation analysis. Multiple studies have demonstrated that the survival time for PTCL is between 150 and 230 days with multi-agent chemotherapy [6-8].

B cell chronic lymphocytic leukemia/small cell lymphoma is a disease characterized by small, mature appearing circulating B cells. The normal counterpart of B cell CLL is not clear, but most likely they are derived from some form of activated B cell. B cell CLL can be diagnosed by identifying more than 5000 circulating small mature B cells/ul (cell size determined by flow cytometry), or if the expansion is less than 5000, by a combination of flow cytometry and clonality testing [3, 9]. While overall this disease appears to have a good outcome, a subset of cases exhibit a significantly more rapid disease course. In particular, Boxer dogs in the U.S. have a short survival, despite the fact that gene expression profile studies suggest that their form of B cell CLL is similar to that in dogs with good outcomes. Studies from our laboratory have shown that Ki-67 levels by flow cytometry may be a simple and inexpensive way to predict clinical course.

Figure 1 summarizes the rough survival times of the different types of canine lymphoproliferative disorders that are currently in the literature.

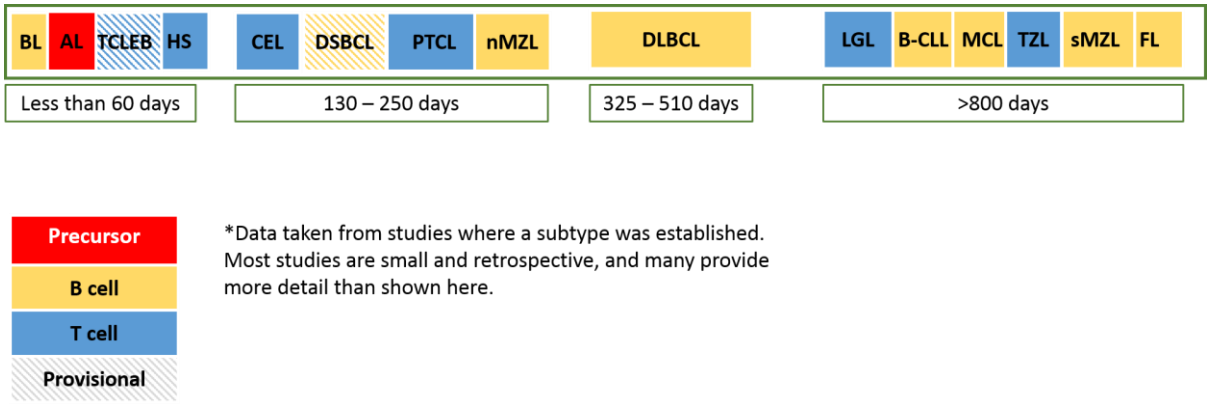

Figure 1: Median survival times of different subtypes of canine nodal lymphoma based on published studies.
BL – Burkitt's; AL – acute leukemia; TCLEB; T cell leukemia of English bulldogs (provisional diagnosis); HS – hepatosplenic lymphoma; CEL – cutaneous epitheliotrophic lymphoma; DSBCL – diffuse small B cell lymphoma (provisional diagnosis); PTCL – peripheral T cell lymphoma; nMZL – nodal marginal zone lymphoma; DLBCL – diffuse large B cell lymphoma; LGL – large granular lymphocytic leukemia; B-CLL – B cell chronic lymphocytic leukemia; MCL – mantle cell lymphoma; TZL – T zone lymphoma; sMZL – splenic marginal zone lymphoma; FL – follicular lymphoma.

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Supplemental References: List of papers used for survival time information in Figure 1.

Acute leukemia: [1-4]
Hepatosplenic lymphoma: [5]
T cell leukemia of English bulldogs: Frankhouse, *in preparation*.
Cutaneous epitheliotrophic lymphoma: [6]
Peripheral T cell lymphoma: [7-9]
LGL leukemia: [10]
T zone lymphoma/leukemia: [9, 11-15]
Burkitt's lymphoma: [13]
Diffuse small cell B cell lymphoma: Hughes, *in preparation*
Nodal marginal zone lymphoma: [16]
Diffuse large B cell lymphoma: [13], Wolf-Ringwall, *submitted.*
Follicular lymphoma: [17]
B cell chronic lymphocytic leukemia: [3, 18]
Splenic marginal zone lymphoma: [19, 20]

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Staging of Canine Lymphoma

STATE OF THE ART OF LYMPHOMA STAGING

Clinical staging in canine non-Hodgkin lymphoma

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The development of clinical staging (CS) schemes has significantly advanced oncologic care. CS concepts were initially based on anatomical characteristics. With the advent of increasingly sophisticated analytical and imaging systems, CS of many tumour types has been considerably refined. Today, CS not only relies on anatomical features, but also on clinical symptoms (e.g. presence (substage B) or absence (substage A) of clinical signs, the differentiation of the tumour (e.g. grade), immunological and histopathological data, leading to the establishment of tumour classification guidelines. CS achieves several important goals in cancer management. These include identification of the optimal therapy, establishment of an accurate prognosis, and precise stratification of patients to enable clinical research and quality assessment. In addition, initial tumour staging in the course of a diagnosis is a prerequisite for accurate restaging of the condition at the end of therapy or documentation of complete remission. As with all malignant tumours, staging of diagnosed non-Hodgkin Lymphoma (NHL) is crucial. However, the development of a CS system for NHL has been challenging. This was, at least in part, due to the heterogeneous nature of NHL subtypes. Lymphoid malignancies are generally classified as leukemia when they primarily involve peripheral blood and bone marrow, and as lymphoma when predominantly involving lymph nodes or other organs. However, some lymphoid malignancies simultaneously exhibit leukemia- and lymphoma-specific features (e.g. T-acute lymphatic leukemia/lymphoblastic lymphoma, T-ALL/T-LBL and chronic lymphocytic leukemia/small lymphocytic lymphoma, CLL/SLL). In addition, malignancies arising from immune cells comprise a wide spectrum of diseases extending from indolent tumours (e.g. T-zone lymphoma, TZL and mucosa-associated lymphatic tissue-lymphoma, MALT-lymphoma) to the most aggressive cancers (e.g. Burkitt lymphoma, BL and acute undifferentiated leukemia, AUL).
The first system widely utilized for CS of NHL was actually developed for staging human Hodgkin disease and is referred to as the Ann Arbor Staging System (AAS). This system is mainly based on anatomical features, yet also takes the presence or absence of systemic symptoms (B-symptoms) into account. Over the years, the AAS also adjusted for the staging of human NHL. The absence of classical Hodgkin's lymphoma in veterinary oncology led to the establishment of a WHO staging (WHOS) system in 1980. However, since the WHOS does not reflect the existence of primary extranodal NHL (e.g. MALT-lymphomas, cutaneous lymphomas, primary diffuse large B-cell lymphoma of the central nervous system, DLBCL-CNS) or some nodal NHL (e.g. splenic marginal zone lymphoma, sMZL) in an appropriate, clinically useful way, it has been suggested to combine both systems for the staging of NHL in animals.

The latter should be first classified according to the WHO classification (WHOC) system, which represents a worldwide consensus on the diagnosis of lymphoid tumours. This system is based on the recognition of distinct disease entities using a multidisciplinary approach and has been updated recently. The current version provides refined definitions of well-recognized diseases, new tumour entities and variants, and emerging concepts contributing to a better clinical understanding of lymphoid neoplasms. The updated WHO classification system takes into account all clinically relevant information, i.e. cell morphology, immunophenotype, clinical topography and behavior, and genetic aspects. Hence, it recommends the performance of morphologic examination, immunophenotyping, antigen receptor gene rearrangement studies (clonality assay, PARR), and other investigations. The WHOC of NHL allows for prediction of the prognosis and selection of the most appropriate therapy for each subtype. If possible, classification should be carried out by an experienced veterinary hematopathologist assessing an excisional biopsy of an affected lymph node or extra lymphatic tumor. Large core-needle biopsies are an alternative in selected cases where excisional biopsy is unfeasible or dangerous (e.g. mediastinal lymphoma). Fine needle aspirates are only sufficient as a starting point for further diagnostic workup. Cytology is inappropriate for differentiating various WHO subtypes or make treatment decisions.

The appropriate CS includes: a careful recording of history and physical examination; appropriate diagnostic imaging of the thorax and abdomen; blood tests (biochemistry panel, complete blood count and blood smear assessment); liver and splenic aspiration and bone marrow aspirate and/or biopsy. However, clear definitions of what constitutes a site of involvement and whether advanced diagnostic imaging (contrast-enhanced ultrasonography (CEUS); computed tomography (CT); positron-emission tomography (PET); and magnetic resonance imaging (MRI) can substitute biopsies in the near future are subject to debates and investigations in human and veterinary oncology. Human patients at high risk for central nervous system involvement usually undergo an MRI and a cerebrospinal fluid-sampling. This method may also be adopted in veterinary oncology. Whether bone marrow aspirate and/or biopsy should be included in CS or not is controversially discussed by different veterinary lymphoma experts. However, collection of a bone marrow biopsy is challenging in so far as enough sample material for immunophenotyping and optimally histological evaluation needs to be obtained. Whether this is achieved by single or multiple punctures probably does not matter. In the past, there was much debate regarding the critical size of a lymph node to be classified as abnormal. By consensus, lymph nodes greater than 1.5 cm in maximum diameter are considered abnormal and assumed to be affected by the disease. Documentation of splenic involvement can be problematic, but feasible by palpation combined with imaging diagnostics, and analysis of organ aspirate. Liver enlargement or abnormal liver biochemistry alone provide not enough evidence for liver infiltration. Liver involvement is usually demonstrated by abnormalities on imaging and optimally by liver biopsy or fine needle aspirate and cytology. Lung involvement is usually documented via imaging. In equivocal cases, analysis of lung aspirates or bronchoalveolar lavage may be necessary. Clinical involvement of the brain can be documented by characteristic MRI abnormalities, but more commonly, primary brain lymphomas require aspiration/biopsy assessment to document their nature. The WHOS also designates patients as either representing substage A or B. Designation as A indicates the absence of any clinical symptom that could be related to lymphoid disease. Substage B is simply defined by the presence of any systemic sign. A recent study used a survey to query veterinary oncologists on the clinical criteria defining the respective substages. Gastrointestinal, constitutional, respiratory, neurologic, metabolic and nutritional parameters were integral in assigning a defined clinical substage. For most factors, a mild to moderate severity of clinical signs was sufficient for substage B designation. However, any independent clinical symptom could be interpreted as substage B making this system less reliable. In human oncology, presence of

B-symptoms is used to refer to systemic symptoms of lymphoma as unexplained fever with temperature greater than 38°C, unexplained weight loss of more than 10% within six months before diagnosis and night sweats. According to our recent results, B-symptoms are of higher prognostic value than substage B in canine nodal diffuse large B-cell lymphoma (DLBCL).
There will be further advances in our ability to evaluate patients with NHL. An obvious possibility is to have different staging systems for different NHL subtypes. For example, the International Prognostic Index has been used for human nodal DLBCL. An adopted system has been developed for patients with follicular lymphoma (FL) to take into account differences between these patients and those with DLBCL. None of the current systems in use for evaluation of patients with NHL refers to the presence of bulky disease. However, the presence of a very large tumour mass is a predictor of poor treatment outcome in human NHL, and some veterinary oncologists take the possible presence of bulky disease into account and treat these sites by radiotherapy (e.g. mediastinal T-LBL). The primary site of origin of a NHL most likely influences the biological characteristics of the malignancy. There is no reason to believe that DLBCL as any other tumour, e.g. canine melanoma, originating at different sites of the body would have the same behavior. Consequently, current classification systems may require further refinement in respect to the localization of the initial primary lesion. The most important factor that will likely change the way we evaluate lymphoma patients is the rapidly expanding knowledge on tumour-specific gene and protein expression. Whatever the future holds, at the present time an accurate CS evaluation is the key to the management of a patient with newly diagnosed NHL. It is important to point out that knowledge is still limited and an individual approach to each patient is dynamically developing.

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Role of Cytology and Flow cytometry in clinical staging

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Lymphoma staging has gained considerable importance in the past years on the way towards patient tailored therapy. The World Health Organization (WHO) staging has been adapted to the canine patient using the Clinical Working Group of European Canine Lymphoma Network recommendations. For diagnosis the WHO classification and/or Flow Cytometry and cytomorphological analysis to define B/T immunophenotype and morphological subtype are required. Histopathologic diagnosis of lymphoma should be achieved performing lymphadenectomy rather than taking core biopsies. For determining the clinical stage, a complete blood count and blood smear evaluation, thoracic and abdominal imaging [x-ray, ultrasound, CT/MRI], cytology of splenic and hepatic aspirates and bone marrow evaluation prior to therapy are desired.
Cytology and flow cytometry are discussed reviewing recent developments in staging and methods for establishing a valid prognosis. Peripheral blood and bone marrow infiltration as well as liver and spleen are mentioned and their prognostic potential is lighted upon. Flow cytometry is already proposed for the detection of infiltrating lymphoma populations in peripheral blood and bone marrow in large B-cell lymphomas (LBCL) (Riondato Res Vet Sci 115 (2017) 288-293) and prognostic cut off values are given for blood and bone marrow infiltrating cells in nodal marginal zone lymphoma (nMZL) (Marconato et al. Vet J 246 (2019) 78-84). The fact that infiltrating B-cells are larger than the remaining reactive population makes staging of these two entities feasible.
It is on future studies to prove the now proposed cut off values and to extend the staging to other entities. Another goal should be to adapt treatment protocols based on the extent of PB and BM infiltration.

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Role of Histopathology, Immunohistochemistry and PARR in clinical staging

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Histopathology including morphological and immunophenotypic assessment is generally considered as essential for the precise subtype diagnosis of canine lymphoma. This is true despite of the fact that in the daily practice cytology and immunocytology are used much more frequently owing to advantages pertaining to easier handling, faster processing and lower costs (Regan et al., 2013). The

WHO classification is the current histological reference system. It has demonstrated a high level of accuracy for the most frequent nodal lymphomas upon use by pathologists with different degrees of experience by allowing classification consistency. In addition, it has the potential to be updated as new knowledge is available (Valli et al., 2011). As for all the most recent, preceding canine lymphoma classification schemes such as the Rappaport classification, the Kiel classification, the working formulation and the updated Kiel classification, which still is used today as an alternative system, it is based on the current human WHO classification. Accordingly, histological diagnosis with immunophenotyping in order to determine B- or T-cell lineage is a minimal requirement for diagnosis of a specific subtype (Valli et al., 2013), and excisional biopsy is the preferred method although incisional or thru-cut biopsy is in many instances sufficient (Zandvliet, 2016). Immunophenotyping with only 2 antibodies (CD3 and CD79alpha or CD20) can result in up to 20% unclassified (including both double positive and double negative) lymphoma cases (Guija de Arespacochaga et al., 2007; Zandvliet, 2016). Interestingly, both cutaneous epitheliotropic T-cell lymphoma and intestinal T-cell lymphoma were shown to be frequently CD20+ (54% and 34%, respectively) (Noland and Kiupel, 2018; Ewing et al., 2019; Matsumoto et al., 2019). Thus, larger panels are more reliable. The antibodies most commonly used for immunophenotyping include CD20, CD21, CD79alpha, PAX-5 for B-cell lymphoma and CD3, CD4 and CD8 for T-cell lymphoma (Zandvliet, 2016). The utility of additional immunohistochemical markers has recently been reported for instance for the differentiation of splenic Marginal Zone lymphoma vs. Mantle Cell lymphoma (Stein et al., 2019). The proposed antibody panel include reagents against Bcl-2, Bcl-6, MUM-1 and Mcl-1 in addition to antibodies identifying B- and T-lineage (CD20, CD3) (Stein et al., 2019). It can be expected that in the future the use of additional markers will contribute to further define an increasing number of entities.

For practical reasons, histology and immunohistochemistry can be considered as of limited value for staging. Interestingly, in a study in diffuse large B-cell lymphoma (DLBCL) in 14 dogs, the mere histological evaluation of lymph node tissue after completion of chemotherapy revealed the frequent finding of a distorted architecture with a reduced number of germinal centers as well as a mixture of small, medium and large lymphocytes present in the cortex with a loss of normal anatomical distribution. Overt DLBCL was observed only in 2 of the cases, but 8 of the remaining dogs eventually relapsed, supporting the limited value of histology for end-staging and the detection of minimal residual disease (Aresu et al., 2014).

In contrast to morphological and immunological methods, molecular tools such as polymerase chain reaction for antigen receptor rearrangement (PARR) amplifying the variable regions of the immunoglobulin genes and the T-cell receptors are useful for differentiating between reactive and neoplastic lymphoid proliferations (Keller et al., 2016; Hwang et al., 2019). Some issues related to false negative results must be taken into consideration for its diagnostic application. A further problem in the daily use is the general lack of information provided by laboratories offering this test about the primer sets and parameters used for interpretation. Apart from that PARR, together with FC, is a tool predestined for use in staging. PARR has been compared to FC for assessment of minimal residual disease in dogs with diffuse large B-cell lymphoma (end-staging), specifically in lymph nodes, peripheral blood and bone marrow (Aresu et al., 2014). PARR was more sensitive than FC in predicting time to relapse, however the combination of PARR and FC was more sensitive than either technique alone in predicting lymphoma specific survival using peripheral blood samples. However, costs might be a factor limiting its use. This might also apply to the highly sensitive monitoring of minimal residual disease, as an extension of PARR, using RT-qPCR with allele-specific oligonucleotide primers and probes designed from neoplastic clones of the individual patients (Sato et al., 2016). The use of more sensitive methods than for instance cytology for staging is potentially

associated with stage migration (i.e., dogs with lymphoma who would have been placed in an earlier stage group [WHO stages I–III] are subsequently moved into a more advanced stage group [WHO stages IV and V]) whose consequences in terms of clinical management and outcome are not well defined yet (Flory et al., 2007).

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Shortcomings of the current practice of staging: the example of DLBCL vs MZL

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In human oncology, the currently used WHO classification provides an international standard for pathologists and oncologists. It represents an evolution of previous classification systems, by integrating morphologic, immunophenotypic, and genotypic features to define subtypes within both the B-cell and T-cell categories. Its strength relies in the identification of disease entities of clinical relevance, having morphologic, antigenic and genetic coherence. Consistent with the experience accumulated in human lymphoma, a future goal in veterinary oncology will be to tailor therapy according to specific diagnostic entities instead of treating canine lymphomas as if they were all the same. To accomplish this, it will be necessary to recognize all of the existing and new lymphoma entities, including their genetic abnormalities and immunologic characteristics.
Appropriate management of dogs with lymphoma begins with a well-timed and accurate diagnosis, taking into account both tumor and patient characteristics.
Pretreatment clinical staging, which is derived from WHO guidelines, accomplishes several important goals: it determines the anatomic extent of lymphoma, allows an accurate prognosis to be given to the owners, and helps directing therapy. The knowledge of lymphoma extension makes it possible to accurately restage dogs at the end of therapy to document type of response. Last but not least, standardized methods for staging are essential to critically assess and compare different therapeutic strategies, as incomplete staging work up impedes the comparison of study results.
Historically, canine lymphoma has been staged according to the WHO guidelines, which defines 5 disease stages. While the prognostic significance has been validated for aggressive B-cell lymphoma, it remains unclear whether stage V disease retains the same relevance for indolent lymphomas, which are typically leukemic at diagnosis and not necessarily associated with a poor outcome.

The studies published over the years have resulted in a considerable increase in knowledge, but also revealed controversies in opinions and beliefs. Many questions remain in the field of diagnostic criteria and staging requirements, which are of direct consequence for the management of dogs.
Doubtless, the different opinions on the minimum criteria for the diagnosis of canine lymphoma do result in an essentially different patient selection in chemotherapeutic protocols and will therefore bias treatment outcome.

1. Cytology versus histology

Traditionally, the diagnosis of lymphoma has relied on the examination of fine-needle aspirates of peripheral enlarged lymph nodes by clinical pathologists. The advantages of cytology include accuracy, minimal invasiveness, rapidity and low costs. Furthermore, flow-cytometric immunophenotyping on lymph node aspirates enhances the diagnostic potential of cytology and provides information on the immunophenotype. The immunophenotype does not impact stage; however, due to its strong association with prognosis, it needs to be determined in the initial work-up.
Despite all advantages of cytology, the obtainment of the correct diagnosis (histotype) is crucial for prognostic and therapeutic considerations, and cannot rely in the majority of cases on cytological details.
In the last years, histopathology has gained attention. Histopathology is the gold standard for diagnosing lymphoma in people, whereas fine-needle biopsy may be appropriate as the only

diagnostic test in the rare patients requiring emergency treatment or those not suitable for curative therapy. Conversely, histopathology is not routinely performed in dogs with lymphoma. According to a survey conducted in 2013, only 28% of veterinary oncologists or veterinarians with a special interest in oncology recommended histopathology. Nevertheless, the value of histopathological analysis has been validated in more recent study. Indeed, with the increasing knowledge about cancer biology, veterinary oncologists must be aware that the complexity of lymphoma classifications and the prognostic importance of architectural assessment in lymphoid neoplasms may advocate lymphadenectomy to confirm and better characterize a primary cytological diagnosis of lymphoma, to accurately determine prognosis and to guide therapy. An incisional biopsy may provide only a glimpse of architecture, limiting interpretation. Therefore, where possible, an intact lymph node should be removed. While the 31 specific disease entities in the WHO classification may at first glance appear overwhelming, it is noteworthy that biologically distinct morphological groups have been identified, possibly having prognostic and therapeutic implications: indolent B-cell, aggressive B-cell, indolent T-cell, and aggressive T-cell lymphomas.

2. Imaging versus imaging plus cytology

Conventional radiology retains an essential role in the care of the dog with lymphoma, from initial diagnosis to monitoring treatment response. Thoracic radiographs should always be performed, as pulmonary (extranodal) involvement may affect prognosis at least in some histotypes.

A basic abdominal investigation of a dog with suspected lymphoma should include ultrasonic and fine-needle aspiration examination of any altered organs, to confirm lymphoma in case of sonographic abnormalities and rule out other possible causes, and to diagnose early lymphoma in case of no sonographic abnormalities. Also, the addition of liver/spleen FNA may determine stage migration to lower stages.

3. Bone marrow evaluation, it is necessary?

The answer to this question is definitely yes, as it affects prognosis and may change the therapeutic approach.

Beside morphology, flow cytometry is a sensitive technique to detect and quantify neoplastic lymphoid cells in the bone marrow, and, by labeling cells with CD34, it can be used to differentiate lymphoma (CD34-) from acute lymphoblastic leukemia (CD34+), disease entities that have a very different prognosis. It has been recently shown that blood abnormalities are not always predictive of bone marrow infiltration, thereby possibly understaging dogs if the marrow is not included in the initial staging work-up. Recently, we have assessed the influence of different levels of bone marrow infiltration, detected by morphology coupled with flow cytometry, on the duration of the first remission and survival in dogs with diffuse large B-cell lymphoma. A significant association was documented between bone marrow infiltration and both disease-free interval and survival time. Also, a cut-off of 3% bone marrow infiltration had prognostic value, as it allowed discriminating between dogs harbouring a poor prognosis and dogs with a more favourable prognosis.

More recently, we have carried out the same study on dogs with nodal marginal zone lymphoma (nMZL). A cut-off of 20% BM infiltration on the basis of FC separated dogs with nMZL in 2 groups with poor and better prognosis.

It is clear that not only bone marrow evaluation is important for prognostic considerations, but it also needs to be put in the context of histotype, and the example of DLBCL versus MZL is a significant example.

Treatment may also be affected by bone marrow infiltration. A small study has evaluated the efficacy of myelo-ablative protocols for the treatment of canine lymphoma with bone marrow infiltration,

showing that the prognosis can be improved. More recently, it has been shown in a large study (including 300 dogs), that chemo-immunotherapy improves outcome for dogs with stage V disease, regardless of the histotype, if the following risk factors are also present (elevated LDH serum level, absence of symptoms, no pre-treatment administration of steroids).
Because bone marrow evaluation is a safe procedure and, if positive, has a profound impact on the prognosis and possibly on the management of the dogs, I routinely add it to the initial staging work-up.

4. End-staging: what is worth it?

In dogs with multicentric lymphoma the measurement of peripheral lymph nodes size after chemotherapy is used to document treatment response. According to the Veterinary Cooperative Oncology Group (VCOG) guidelines, clinical CR is defined as the regression of all affected peripheral LNs to a size considered normal by physical examination using LN palpation and calipers. Following clinical remission, the residual population of tumor cells can be referred to as the minimal residual disease (MRD), which is implicated as the source of tumor relapse. Therefore, measurement of MRD can provide information regarding the presence of neoplastic cells even in the clinical CR phase.
Several techniques, including FC and polymerase chain reaction amplification of antigen receptor genes (PARR), have been used to detect MRD in LN, peripheral blood (PB) and bone marrow (BM) samples in dogs with LBCL. Molecular techniques for detection of MRD in PB samples during or following treatment can be used as an objective parameter for the determination of treatment efficacy and to select dogs that might benefit from consolidation chemotherapy despite clinical CR. However, the universal primers previously designed for PARR showed a low sensitivity for MRD evaluation.
We have recently shown that MRD assessment by FC on LN aspirates is a useful tool for assessing the presence of subclinical disease in dogs with large B-cell lymphoma treated with chemo-immunotherapy. Relapse occurrence could be efficiently predicted through FC prior to clinical relapse diagnosis, and the value >0.5% was associated with early recurrence.

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Abstracts of Poster and Oral Presentations

A01 Focal Adhesion Kinase (FAK) inhibition potentiates the direct cytotoxic effect of an anti-major histocompatibility complex class II (MHC-II) antibody towards canine B cell leukemia/lymphoma lines (Poster)

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Introduction. Small molecule focal adhesion kinase inhibitors are emerging anti-tumor agents with *in vitro* proven action towards various human non-lymphoid tumor cell lines. Little is known about the activity of these compounds towards lymphoid malignancies and in particular canine leukemia/lymphoma cell lines.

The aim of this study was to assess if treatment of three canine B cell lymphoma/leukemia cell lines with FAK inhibitors alone or in combination with murine monoclonal anti-DLA-DR antibody B5 can induce and/or potentiate programmed cell death.

Materials and Methods. CLBL1, CLB70 and GL1 B-cell lines representing canine hematologic malignancies were treated for 24 hrs with anti-MHC-II DR monoclonal antibody B5 (10 g/ml) alone or in the presence of two FAK inhibitors (FAK14 and PF-573271) at concentrations ranging from 0.125 to 2 M. An MTS assay was used to evaluate cell proliferation. Apoptosis induction was assessed by CellEvent® Caspase-3/-7 Green Detection Reagent. Statistics was performed using an unpaired two-tailed Student's test, $p<0.05$ values were considered significant.

Results. FAK14 and PF-573271 inhibitors at the highest tested concentrations only minimally affected cell proliferation and apoptosis rates of canine cell lines. However, when used in combination with B5 antibody, they potentiated antibody-induced cell death of MHC-II expressing cell lines. Average percentages of cells displaying active caspase-3/-7 after 24 hrs of antibody alone treatment reached 35.3%, whereas combination treatment with 0.5 M and 1.0 M of FAK14 increased this value to 49.8%* and 60.4%*, respectively (*p<0,05). Similar values were also scored for PF-573271 inhibitor.

Conclusion. Anti-MHC-II antibody crosslinking of canine B cell leukemia/lymphoma lines triggers both pro-survival and pro-apoptotic signaling pathways. FAK inhibitors counteract an important pro-survival signaling pathway relating to integrin activation and concomitantly sensitize these cells to MHC-II induced apoptosis. Therefore, the present study highlights a novel target to consider for the treatment of canine B cell leukemia/lymphoma.

A02 Evaluation of a CHOP rescue protocol with a maintenance phase for canine relapsed large-cell lymphoma (Poster)

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Introduction. The aim of this retrospective study was to evaluate a CHOP rescue protocol with a maintenance phase (MP) for dogs with large-cell lymphoma at time of first relapse.

Material and methods. Medical records from 2003 to 2018 were reviewed. Dogs with large-cell lymphoma treated with a CHOP rescue protocol with MP at first relapse were evaluated. The first phase consisted of a 12-week CHOP protocol (Table 1). MP contained two alkylating agents per os (PO), a vinca alkaloid, an anthracycline or anthracendione intravenously (IV) and prednisolone PO. Treatment intervals in MP were every 2 weeks (Table 2). MP was given life-long or until progression. Progression free interval (PFI) and toxicity (VCOG criteria) were assessed.

Table 1. First phase

	Week			
Drug	1	2*	3*	4*
L-Asparaginase SC	X			
Vincristine IV		X		
Cyclophosphamide IV			X	
Doxorubicin IV				X
Prednisolone PO	X	X	X	X

*repeat 4 cycles

Table 2. Maintenance phase

	Week		
Drug	14*	16*	18*
Cyclophosphamide PO	X		
Chlorambucil or Melphalan PO		X	
Anthracycline, Anthracendione or Vinca alkaloid IV			X
Prednisolone PO	X	X	X

*repeat life-long

Results. Twenty-three dogs were included. Immunophenotype was known in 6 dogs, 5 had a B-cell lymphoma and 1 a T-cell lymphoma. Twenty-one of 23 dogs (91%) were in complete remission (CR) at start of MP. MP was initiated after median 7 weekly treatments at clinician's discretion. Sixteen of 23 dogs (70%) completed at least one cycle of MP. Adverse events were generally mild. During the first phase, 1 septic event and 2 hospitalizations due to gastrointestinal side effects occurred. No septic events or other hospitalizations occurred during MP. Median PFI was 252 days (n=22, range: 28-887 days).

Conclusions. The protocol was well tolerated and is a treatment option at first relapse for dogs with large-cell lymphoma.

A03 The effect of xanthohumol derivatives on apoptosis induction in canine lymphoma and leukemia cell lines (Poster)

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Introduction. Xanthohumol is a prenylflavonoid isolated from spent hops, which has various biological properties such as antioxidant, anti-inflammatory, antibacterial, antiviral and antiproliferative ones. We synthetized eight xanthohumol derivatives obtained by chemical modification or biotransformation and checked their antiproliferative properties in canine leukemia/lymphoma cell lines.

Materials and Methods. The compounds were tested at final concentrations range of 0.1 – 30 μM for 48 hrs**.** Evaluation of the viability of the cells was performed using an MTS test. Induction of apoptosis was determined by Annexin V and propidium iodide (PI) staining using flow cytometry. Expression of several pro- and anti-apoptotic proteins, influencing the efficacy of chemotherapy (Bcl-2, Bax, BID, Mcl-1, p53 and PARP) was analyzed using Western blotting. We used four different canine cell lines: CLBL-1 (B-cell lymphoma), CLB70 (B-cell leukemia), GL-1 (T-cell leukemia} and CL-1 (T-cell lymphoma).

Results. All eight drugs tested exerted concentration-dependent cytotoxicity in the selected canine lymphoma/leukemia cell lines. Three compounds markedly decreased the viability of all cell lines with IC_{50} in the range of 0.5 to 8 □M. The remaining compounds were less effective especially against T-cell lines. Double staining of treated cells with Annexin V/PI revealed that dying cells were mostly in late apoptosis. Western blot analysis showed cleavage of PARP, thus confirming apoptotic cell death, and a decreased expression of Bcl-2, while the levels of all other proteins remained unchanged.

Conclusions. Canine lymphoma and leukemia cell lines of both B- and T-cell lineage are sensitive to xanthohumol derivatives which act through an apoptotic cell death mechanism. These drugs either used alone or in combination with other therapies may be useful for the treatment of canine leukemia/lymphoma.

A04 Leukocyte ratios as prognostic indicators in canine peripheral T-cell lymphoma (Poster)

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Introduction. Peripheral T-cell lymphoma (PTCL) encompasses a group of aggressive lymphomas. In humans, leukocyte ratios are helpful in refining the cancer prognosis. However no study has yet focused on leukocyte ratios in canine PTCL.
The aim of this retrospective analysis was to evaluate neutrophil-to-lymphocyte (NLR), neutrophil-to-eosinophil (NER), neutrophil-to-monocyte (NMR), lymphocyte-to-monocyte (LMR), eosinophil-to-monocyte (EMR) and eosinophil-to-lymphocyte (ELR) ratios as prognostic factors in canine PTCL.
Materials and Methods. A total of 21 nontreated patients with pretreatment blood analysis (Siemens Advia 2120™, Sysmex XT-2000iV) were included. If necessary, a microscopic correction was performed. Leukocyte ratios were calculated from their absolute concentrations. Cut-off values of 3.5, 25.0 and 1.2 were set for NLR, NER, and LMR, respectively based on previous studies in canine lymphoma and mast cell tumour. Cut-off values of 8.0, 0.1 and 0.11 for NMR, EMR and ELR, respectively were adopted from previous human studies. All patients were treated by chemotherapy (CHOP or LOPP). Survival curves were analyzed with the Kaplan – Meier method and differences assessed by the log-rank test.
Results. Median values ($x10^9$/L) for neutrophils, lymphocytes, monocytes and eosinophils were 6.74, 1.66, 0.65 and 0.13, respectively. All ratios were independent from age, weight, sex, lymphoma grade, stage or substage. Initial treatment response was negatively linked to high NER (p=0.002) and low ELR (p=0.03). No differences in progression-free survival (PFS) nor lymphoma-specific survival (LSS) were observed in NLR, LMR, EMR or NMR. High NER was associated with inferior PFS (43 vs. 152 days, p=0.004) and LSS (94 vs. 210 days, p=0.03). Low ELR was linked to shorter PFS (33 vs. 144 days, p=0.003) and LSS (68 vs. 205 days, p=0.004).
Conclusions. In our group of PTCL patients only NER and ELR indicated inferior survival. However larger prospective studies on different WHO lymphoma types with standardized treatment protocol are warranted.

A05 Occurrence of vector-borne pathogens in canine nodal lymphomas (Poster)

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Introduction. Lymphoma is a common malignancy in dogs, but little is known about its infectious etiology. In humans, the detection of DNA of vector-borne diseases (VBD) in non-Hodgkin lymphomas (NHL) has raised the hypothesis that VBD could be causally related to NHL development. Several veterinary reports suggested a possible link between VBD and the tumorigenesis of canine nodal NHL (nNHL).
The aim of our prospective pilot study was to assess the presence of DNA of vector-borne diseases in canine nodal lymphomas classified according to the WHO classification.
Materials and methods: The study, conducted in 2018, included 36 dogs with nNHL distributed within 11 subtypes. Inclusion criteria were no oncologic pretreatment, including corticoids, and a complete clinical staging. History of *tick* infestation and VBD was ascertained through owner´s interviews. Real-time PCR analyses for *Anaplasma platys and phagocytophilum, Babesia canis, Borrelia burgdorferi spp., Ehrlichia canis, Hepatozoon canis, Leishmania infantum and Candidatus Neoehrlichia mikurensis* were performed from peripheral blood in all and from lymph node in 13 dogs.
Results: Twenty-one dogs had a tick infestation in the last three months and five had a VBD history (three *Anaplasma phagocytophilum*, two *Babesia canis*). VBD-DNA was not detected by real-time PCR in any of the samples.
Conclusion: This study found no evidence that VBD are directly involved in common types of canine nNHL. It was limited by the detection of only DNA without serology. Follow-up studies on larger numbers of patients and detecting VBD-DNA in other organs and including also serology are in progress.

A06 Are B-symptoms more valuable than substage B to predict prognosis in canine nodal diffuse large B-cell lymphoma? (Oral)

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Introduction. B-symptoms (BS) refer to systemic symptoms of lymphoma in human patients as fever, weight loss and night sweats. In canine lymphoma, substage B (SSB) is used to describe any symptom observed.
The aim of this retrospective study was to compare the value of SSB and BS to predict treatment response and survival in canine nodal diffuse large B-cell lymphoma (nDLBCL).
Materials and Methods. Fifty-five dogs with nDLBCL diagnosed by histopathology and immunohistochemistry and treated with standardized CHOP chemotherapy between 2008-2018 were included. BS were defined as weight loss greater than 10% of normal weight, unexplained fever and presence of unexplained tachypnea in rest. SSB was defined as any symptom but lymphadenopathy. Survival curves were analyzed with the Kaplan – Meier method and differences assessed by the log-rank test.
Results. BS were present in 20/55 (36%) and SSB in 39/55 (71%) patients. No significant association between BS or SSB and weight, sex, breed, stage and lymphoma grade were found. Treatment response after induction chemotherapy was negatively associated with both SSB (P=0.02) and BS (P=0.001). BS decreased significantly progression free survival (PFS) (95 vs. 330 days, P=0.001) and lymphoma specific survival (LSS) (160 vs. 462 days, P=0.001). SSB showed a tendency toward shorter PFS (160 vs. 240 days, P=0.18) and LSS (188 vs. 332 days, P=0.23).
Conclusions. According to our results, BS appear to be a better prognostic factor than SSB in canine nDLBCL. Prospective studies assessing BS in a larger cohort of patients and in other common lymphoma types are warranted.

A07 Potential and limitations of Clonality Testing – experiences with clinical cases (Poster)

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Introduction. Clonality testing is neither a standalone tool for diagnosing lymphoma nor a method to discriminate between B- and T- cell lymphoma. It is helpful when morphologic techniques fail to differentiate a reactive lymphocytic population from neoplasia.
In this retrospective study we investigated the indications for clonality testing and describe PCR-results compared to morphology.
Materials and Methods. In four years, 838 cytologic samples of canine lymphoid tissue were submitted for clonality testing. All slides were evaluated by microscopy. Images were taken for documentation of cellularity and sample quality.
Results. Out of 838 cytology samples, 307 (36%) showed inconclusive morphologic results. Among the remaining 531 (64%) cases, 46 (8.6%) were non-diagnostic, 418 (78.7%) featured morphologically clear-cut lymphoma and reactive hyperplasia (45, 8.5%), or exhibited no assignable cell lineage (22, 4.1%). Within the 45 cases of reactive hyperplasia 15% showed a false positive PCR-result. 15% of cases morphologically consistent with lymphoma, showed a false negative result. Within the 307 morphologically inconclusive samples, 85 (27.6%) showed a polyclonal result, 101 (32.8%) cases were clonal for TRG, 46 (14.9%) clonal for IGH, and five (1.6%) showed clonality for both, TRG and IGH. Finally, three cases (0.9%) displayed a pseudoclonal result, 55 (17.9%) were non-diagnostic due to low DNA concentration or poor DNA-quality and 12 (3.9%) were inconclusive, showing no evaluable traces.
Conclusions. A high percentage of samples were submitted despite a clear cut morphologic diagnosis of lymphoma was possible. However, also in these cases, 15% gave a false negative result, which corresponds to the previously reported sensitivity of 0.86. In the group where morphology suggested a reactive lymphocyte population, 15% showed a false positive result, matching reports where specificity was 0.85. PCR-results have to be interpreted in the light of clinical presentation, immunophenotyping and histopathology to finalize a diagnosis of lymphoma.

A08 Clinicopathological characteristics and prognostic factors for canine multicentric non-indolent T-cell lymphoma: 107 cases (Oral)

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Introduction. Canine lymphoma, as the most common hematopoietic malignancy, encompasses a group of heterogeneous diseases. Even within the T-cell immunophenotype differences in clinical presentation and responses to treatment exist.
The aim of this retrospective study was to determine outcomes and prognostic factors of dogs with multicentric non-indolent T-cell lymphoma (TCL) receiving either lomustine based (70%) or non-lomustine based (30%) chemotherapy.
Materials and Methods. Medical records of dogs diagnosed with non-indolent multicentric TCL from three referral centers in the UK were reviewed. Information regarding signalment, blood work, method of diagnosis, immunophenotype, staging and treatment outcome was recorded. Kaplan-Meier survival curves were depicted and estimated median survival times reported. Predictors of progression-free survival (PFS) and overall survival (OST) were assessed using univariable and multivariable Cox regression analyses. Variables with P-values <0.1 in the univariable analysis were included in the multivariable analysis.
Results. Labrador 19/107 (18%), Boxer 14/107 (13%), crossbreed 14/107 (13%) and Dogue de Bordeaux 9/107 (8%) were most often affected. Eighty-six percent had substage b, 77% had mediastinal involvement, 15% had suspected bone marrow involvement and 12% had other extra-nodal sites of disease. The overall response rate to induction therapy was 80%; inclusion of procarbazine in the induction protocol (P=.042), neutrophil blood concentration below $8.7x10^9$/L (P=0.006) and mitotic rates below 10 per 5 hpf on cytology (P=0.013) were associated with better response rates. Median PFS for the first remission was 105 days (range 1-1677); lack of expression of CD3 on flow cytometry (P<0.0001) and pretreatment with steroids (P=0.012) were associated with shorter PFS. Median OST was 136 days (range 1-1677); co-expression of CD79a (P=0.002), lack of CD3 expression on flow cytometry, presence of anemia (P=0.007) and monocytopenia (P=0.002) were predictive of shorter OST.
Conclusions. This study identified new possible prognostic factors for canine multicentric non-indolent T-cell lymphoma, an aggressive lymphoma subtype.

A09 Usefulness of 6–Colour Multiparameter Flow Cytometry in Canine Lymphoma Phenotyping (Poster)

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Introduction. Flow cytometry (FC) is a critical tool in the objective diagnosis and further characterization of lymphoma. Features such as expression of cell surface markers or cell size can provide important prognostic information. In many laboratories, immunophenotyping of canine leukocytes is usually performed using 2-colour or 3-colour FC.
The aim of this study was to introduce in praxis a compatible 6–colour multiparameter FC protocol for routine lymphoma immunophenotyping using commercially available monoclonal antibodies.
Materials and methods. For 6-colour FC, directly conjugated (CD3-FITC, CD8-AF700, CD25-eFluor660, CD21-PE) as wells as unconjugated (CD4 and γδTCR) anti-dog monoclonal antibodies and appropriate conjugates (PeCy7 and BV421) were used in optimal dilution. Furthermore, fluorescent minus one controls were prepared for compensation set-up. Fifteen samples of peripheral blood or of fine-needle aspirates of peripheral lymph nodes from dogs with suspected lymphoproliferative disease were stained using 2-colour and 6-colour protocols and analysed with a flow cytometer LSRFortessa operated with BD FACSDiva software v. 6.2 (BD Biosciences) to compare the results.
Results. From all tested samples, 9 were diagnosed as B-cell and 6 as T-cell lymphoma (including 3 T-zonal and 2 γδ-TCR lymphoma). No spectral overlaps or nonspecific binding of the used fluorochromes were detected.
Conclusions. Six-colour FC is a powerful method to increase the accuracy of lymphoma phenotyping by simultaneous staining of lymphocyte markers with six specific antibodies in one test tube. When compare to 2-colour FC, 6-colour approach proves to be helpful mainly in case of aberrant phenotypes and in small cell types of lymphoma, where neoplastic cells are hardly differentiable from non-neoplastic lymphocytes. Moreover, lesser total amounts of samples and reagents are used.

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A10 Flow Cytometric Characterization of Lymphoma in Dogs from the Czech Republic – A Retrospective Study (Poster)

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Introduction. Lymphoma is the most common hematologic malignancy in dogs. The diagnosis of lymphoma in dogs is based mainly on morphological assessment (histology, cytology), immunochemistry, flow cytometry and PCR for antigen receptor rearrangements (PARR). Flow cytometry is a very precise and accurate method to determine the immunophenotype of neoplastic cells and can provide important information regarding prognosis and response to therapy.
The aim of this retrospective study was to determine the relative frequencies of various lymphoma subtypes in lymph node aspirates from 112 dogs with suspected lymphoproliferative disease using flow cytometry.
Materials and Methods. Results of flow cytometry examinations performed between January 2012 and December 2018 at the VRI (n=112) were included. The following monoclonal antibodies were used for two or one colour flow cytometry: CD45, CD3, CD4, CD8, γδ-TCR, CD79α, CD21, MHCII, CD45RA and CD90.
Results. B-cell lymphoma was diagnosed in 78/112 (70%) and T-cell lymphoma in 34/112 (30%) cases. All cases of B-cell lymphoma were positive for CD79α and CD45 and all except 4 samples were positive for CD21. Of the CD79α+/CD45+/CD21+ tumors 48 were MHCII+, 6 were MHCII- and 20 lymph node aspirates expressed variable levels of MHCII. Of the T-cell lymphomas, 4 samples each were CD4+ or CD8+, and 3 samples expressed the γδ-TCR phenotype. Four aspirates were immunophenotypically consistent with T-zone lymphoma (CD45-, CD3+, CD21+). Co-expression of CD3 with CD79α was detected in 11 cases. In the remaining 8 samples predominance of CD3+ cells with different expression of other T-cells markers were found.
Conclusions. In accordance with other studies, we confirmed a higher frequency of B-cell vs. T-cell lymphoma in the dog and frequent occurrence of different phenotypes in T-cell lymphomas. In a significant portion of B-cell lymphomas MHCII was variably expressed.

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A11 Tel-eVax: a genetic vaccine targeting telomerase for treatment of canine lymphoma (Poster)

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Introduction. Tel-eVax, a genetic vaccine targeting dog telomerase (dTERT) and based on Adenovirus (Ad)/DNA-Electro-Gene-Transfer (DNA-EGT) technology combined to COP therapy has been previously shown to induce a strong immune response and increase overall survival (OS) of dogs with multicentric Diffuse Large B-cell Lymphoma (DLBCL).
The objectives of this study were: 1) to clinically validate a new device for veterinary electroporation called Vet-ePorator™, based on Cliniporator™ technology (currently approved in Europe for electrochemotherapy applications and adapted to EGT); 2) to combine Tel-eVax with a 27-week Madison Wisconsin CHOP protocol for the treatment of DLBCL and compare OS with historical controls from the same geographical area treated with CHOP.
Materials and Methods. Seventeen dogs affected by DLBCL were vaccinated using two Ad vector injections (Prime phase) followed by DNA-EGT (Boost phase) by means of a Vet-ePorator™ device and concomitantly treated with CHOP. The immune response was measured by previously described ELISA assays using a pool of peptides. The vaccinated animals were closely monitored for body weight, temperature and abnormal values in hematological parameters to monitor signs of toxicity and/or to detect indications of autoimmunity.
Results. No significant adverse effects of Tel-eVax were observed. The OS of vaccine/CHOP animals was 64.5 weeks (range: 47.1-87.05), in line with the previous study. Sixty-seven percent of assessable dogs developed antibodies against the immunizing antigen. An indirect comparison between the 17 patients treated in this study with the dog cohort of lymphoma patients described by Wilson-Robles et al. (2017) in the same geographical area (452 and 244 days, respectively) suggests a ~ 2-fold OS increase, in line with our previous findings.
Conclusions. Tel-eVax in combination with CHOP is safe and immunogenic in lymphoma canine patients. These data confirm the therapeutic efficacy of dTERT vaccine and hold promise for the treatment of dogs affected by other cancer types.

A12 RNA sequencing of canine cutaneous epitheliotropic lymphoma and immune-mediated dermatoses reveals major differences in cell-matrix adhesion and ribosomal proteins (Oral)

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Introduction. Canine cutaneous epitheliotropic T-cell lymphoma (CETL) shares several clinical, histomorphological and cellular features with immune-mediated T-cell predominant dermatoses (IMD), albeit substantially differing in prognosis. The pathogenesis of CETL is poorly understood. Moreover, diagnostic discrimination of early CETL from IMD may be challenging.
This study aimed to uncover pathogenetic differences between CETL and IMD by investigating their transcriptome profile.
Materials and Methods. RNA from archival canine skin biopsies (6 CETL and 9 IMD cases, diagnosis based on clinical history, histopathology and T-cell clonality) was subjected to 100bp single-end sequencing on an Illumina HiSeq3000. DESeq2 was used for principal component (PCA) and differential gene expression analysis (FDR<0.01, IMD as control). Pathway analysis was performed on upregulated genes using the Ingenuity Pathway Analysis Tool and KOBAS 3.0. The expression of selected genes was validated by RT-qPCR (*CD5, FOXP3, ITGB7*, *IL2RB, ILK, METAP1D, MNF1*, *TCF7*) and by immunohistochemistry (FOXP3, TCF7).
Results. Five hundred and three genes were upregulated, while 4986 were downregulated in CETL compared to IMD. PCA demonstrated a separation of CETL from IMD cases with respect to gene expression. Focal adhesion and ribosomal protein pathways were enriched in the CETL group. RT-qPCR confirmed the sequencing results for 6 out of 8 genes tested. Immunohistochemical differences were significant with limited diagnostic value.
Conclusions. The hypothesized transcriptome differences between CETL and IMD cases could be confirmed. Several differentially expressed genes have previously been reported in human cutaneous lymphoma studies. In canine CETL, genes coding for proteins involved in cell-matrix interactions and in the ribosomal machinery are apparently upregulated.