ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

CANINE INDOLENT LYMPHOMA – A COMPREHENSIVE REVIEW

Lua Pereira Férin

Coimbra, julho de 2023

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Aluna do Mestrado integrado em Medicina Veterinária

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List of Abbreviations

BM – bone marrow
CHOP – Cyclophosphamide-Hydroxydaunorubicin-Vincristine and Prednisolone
ciL – canine indolent lymphoma
cL – canine lymphoma
FL – follicular lymphoma
FNB – fine-needle biopsy
HSPPCs-HA – heat shock proteins purified from the dogs tumors
IHC – immunohistochemistry
iL – indolent lymphoma
Ln – lymph node
LPL – lymphoplasmacytic lymphoma
LSS – lymphoma specific survival
MCL – mantle cell lymphoma
MI – mitotic index
MST – median survival times
MZL – marginal zone lymphoma
nMZL – nodal Marginal Zone Lymphoma
PARR – polymerase chain reaction for antigen receptor rearrangements
PB – peripheral blood
PCR – polymerase chain reaction
SCL – small cell lymphoma
ST – survival time
SLL – small lymphocytic lymphoma
sMZL – splenic Marginal Zone Lymphoma
TCRBCL – T-cell rich B-cell lymphoma
TTP – time to progression
TZL – T-zone lymphoma
WHO – World Health Organization

Canine indolent lymphoma – a comprehensive review

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Abstract

Approximately 5 to 29% of canine lymphoma are indolent lymphomas. These lymphomas are classified as low grade, associated with small cells, which could be B or T-cell types. They have a low mitotic index and characteristically progress slowly. The complete aetiology of canine lymphoma seems unclear, but several causes could be established, both external and internal, namely the type of environment (industrial areas and polluted sites) and genetic factors.

According to the World Health Organization classification, there are different subtypes of indolent lymphomas: marginal zone lymphoma, follicular lymphoma and mantle cell lymphoma, T-zone lymphoma, small lymphocytic lymphoma, lymphoplasmacytic lymphoma, T-cell rich B-cell lymphoma and intestinal small cell lymphoma. Recent studies have suggested that T-zone lymphoma is the most common subtype of indolent lymphomas in dogs.

Despite advanced stages of the disease, dogs with indolent lymphomas maintain normal activity and appetite levels. There are several diagnostic techniques described to establish a specific diagnosis of lymphoma in canines, with flow cytometry of fine needle aspirates gaining popularity as a first-line analysis technique.

Treatment approaches for indolent lymphomas should be tailored to the histological subtype, as there is no universal therapeutic approach. The primary goal of therapy is disease control (stable disease or partial response), as complete remission is rare in indolent lymphomas. Chemotherapy, immunochemotherapy, and surgery have varying recommendations depending on the specific subtype of indolent lymphoma. Overall, studies have shown that dogs with indolent lymphoma can live for years without aggressive chemotherapy. However, dogs with indolent low-grade lymphomas have a survival advantage over dogs with intermediate and high-grade lymphomas. Furthermore, research indicates that dogs with indolent lymphomas experience prolonged survival times, often without treatment.

Due to the limited research on this disease in veterinary oncology, further studies are needed to enhance the efficacy of diagnosis and treatment, ultimately improving the quality of life for affected dogs.

Keywords: Aetiology, diagnosis, dog, indolent lymphoma, subtypes, treatment and survival.

Resumo

Aproximadamente 5 a 29% dos linfomas caninos são linfomas indolentes. Estes linfomas são classificados como baixo grau, associados a células pequenas, que podem ser células do tipo B ou T. Este tipo de linfoma apresenta um índice mitótico baixo e caracteristicamente progridem lentamente. A etiologia completa do linfoma canino é pouco clara, mas podem ser estabelecidas várias causas, tanto externas como internas, nomeadamente o tipo de ambiente (áreas industriais e locais poluídos) e fatores genéticos.

De acordo com a classificação da Organização Mundial de Saúde, existem diferentes subtipos de linfoma indolente: linfoma da zona marginal, linfoma folicular, linfoma de células do manto, linfoma da zona T, linfoma linfocítico pequeno, linfoma linfoplasmocítico, linfoma de células B rico em células T e linfoma intestinal de células pequenas. Estudos recentes sugerem que a incidência do linfoma da zona T é o subtipo mais comum de linfomas indolentes em cães.

Os cães com linfomas indolentes apresentam níveis normais de atividade e de apetite, mesmo em fases avançadas da doença. Existem várias técnicas de diagnóstico para realizar um diagnóstico específico deste tipo de linfoma em caninos, sendo que a citometria de fluxo por aspiração por agulha fina tem gradualmente vindo a ser a mais utilizado como técnica de primeira linha de análise.

A abordagem terapêutica do linfoma indolente deve ser feita de acordo com o subtipo histológico, não existindo uma abordagem terapêutica universal. O principal objetivo da terapêutica é o controlo da doença (doença estável ou resposta parcial), uma vez que a remissão completa é pouco frequente nos linfomas indolentes. A quimioterapia, a imunoquimioterapia e a cirurgia têm recomendações diferentes consoante o subtipo de linfoma indolente. Em geral, os estudos mostraram que os cães com linfoma indolente podem viver anos sem quimioterapia agressiva e, muitas vezes, não é alcançada uma resposta completa à quimioterapia. No entanto, os cães com linfomas indolentes de baixo grau têm uma vantagem de sobrevivência em relação aos cães com linfomas de grau intermédio e elevado. Além disso, a investigação demonstrou que os cães com linfomas indolentes podem apresentar tempos de sobrevivência prolongados, muitas vezes sem tratamento.

Devido à investigação limitada desta doença em oncologia veterinária, é necessária mais investigação nesta área para aumentar a eficácia do diagnóstico e do tratamento, de modo a melhorar a qualidade de vida dos cães afetados.

Palavras-chave: Cão, diagnóstico, etiologia, linfoma indolente, subtipos, tratamento e sobrevivência.

1. Introduction

Cancer is considered the biggest public health problem worldwide (Siegel et al., 2023). The incidence of cancer in domestic animals has been increasing (Modiano & Kim, 2020; Sarver et al., 2022), attributed to longer life expectancies and advancements in veterinary medicine (Dobson, 2011).

Lymphoma represents a group of neoplasms originating in lymphocytes and accounts 83% of hematopoietic neoplasms and 7 to 24% of canine neoplasms (Vail et al., 2020). According to the authors the estimated incidence ranges from 13 to 114 cases per 100,000 dogs at risk, and according to age, it is 1.5 per 100,000 in dogs less than 1 year old, and 84 per 100,000 in dogs with 10 years old (Vail et al., 2020).

The World Health Organization (WHO) classification system categorizes lymphoma based on phenotype, cell morphology, tissue architecture, location, and its clinical course. This classification distinguishes the disease between aggressive or indolent lymphoma (iL) (Valli et al., 2011). It can also be based on their anatomical form, with the multicentric form being the most common, followed by gastrointestinal, mediastinal and cutaneous forms (Vail et al., 2020). Primary extranodal forms, such as those affecting bladder, eyes, central nervous system, nasal cavity, and heart, are rare (Vail, 2017). Most canine aggressive lymphomas are classified as high grade, characterized by presenting large lymphocytes, have a high mitotic index (MI), and aggressive biological behaviour (Valli et al., 2013).

Approximately 5 to 29% of canine lymphoma (cL) are iL classified as low grade and are associated with small cells and a low MI (Flood-Knapik et al., 2013; Vail et al., 2020). These studies highlighted that characteristically the ciL progress slowly and are associated with long survival times (ST), even with limited or no treatment, but they remain incurable diseases. Dogs with this type of lymphoma usually preserve normal activity and appetite levels even during advanced stages of the disease (Vail et al., 2020). According to the WHO classification, the following subtypes of iL are defined: marginal zone lymphoma (MZL), follicular lymphoma (FL), mantle cell lymphoma (MCL), T-cell rich B-cell lymphoma (TCRBCL), T-zone lymphoma (TZL), small lymphocytic lymphoma (SLL), lymphoplasmacytic lymphoma (LPL) and intestinal small cell lymphoma (SCL)(Flood-Knapik et al., 2013; Valli et al., 2017).

Given the lack of review studies providing a comprehensive overview of research on iL, conducting a review study on this topic is crucial. Such a study would contribute to a better understanding of the diagnosis, treatment, and survival of dogs with iL, a disease that remains poorly described, but has an increasing incidence over time.

Therefore, the objective of this study is to analyse and summarize published research on iL in dogs, focusing on its aetiology, characterization of iL subtypes, diagnosis, treatment, and survival, and to draw conclusions based on the available literature.

2. Aetiology

The complete aetiology of cL remains unclear, but several causes have been identified as potentially related to the development of cL. These causes can be both external and internal, including, for example, environmental factors, genetic and immune factors (Ruple et al., 2017; Zandvliet, 2016).

2.1. Environmental factors and Geographic

Exposure to specific environmental factors have been associated with an increased risk of developing cL, such as living near waste incinerators, radioactive or polluted sites, living in industrial zones and contact with (domestic) chemicals, and exposure to magnetic fields (Zandvliet, 2016). Oxidative stress or radiation, which can induce a failure in repair DNA damage pathways, are also known to increase the risk of developing neoplastic diseases (Thamm et al., 2013).

Research in this area has shown variations in the geographical distribution of cL. In the USA, the TZL subtype has a higher incidence in East North Central and Northeast regions. However, it should be noted that this study only included golden retriever dogs (Ruple et al., 2017).

2.2. Immune system

Although a dysfunctional immune system has been proposed to play a role in lymphomagenesis, there is currently limited significant data supporting this relationship (Zandvliet, 2016). Studies in dogs with cutaneous lymphoma (*mycosis fungoides*) have identified an augmented risk for dogs diagnosed with atopic dermatitis (Zandvliet, 2016).

2.3. Age and indolent lymphomas

Some authors suggest that age could be related to the development of specific iL subtypes. According to several studies, it has been found that the likelihood of developing TZL increases with age. For example, dogs aged 11 years old, 9-10 years old, and 7-8 years old had 16 times, 8 times, and 4 times, respectively, higher chances of developing TZL compared to younger dogs (Ruple et al., 2017).

Dogs with MZL have a median age of 9 years (range 2.5-12.7) while dogs with FL have a median age of 4.2 years (range 1.6-10.8) at diagnosis (Flood-Knapik et al., 2013). Small lymphocytic neoplasms can occur in younger animals but is more commonly observed in mature dogs (10–15 years of age) (Valli, 2007). Lymphoplasmatic lymphoma affects many dogs of various ages, ranging from 2.5 to 14 years (mean of 3.4 years) (Valli, 2007).

2.4. Breed and other factors

Comazzi et al. (2018), Flood-Knapik et al. (2013), Mizutani et al. (2016a), Seelig et al. (2014), Valli et al. (2006) described that golden retrievers have a high prevalence of TZL (outside the Europe). Moreover, other studies (Comazzi et al., 2018; Harris et al., 2018; Martini et al., 2015, 2016) did not find a high prevalence of TZL in golden retrievers in Europe. Other breeds, such as shih-tzu, golden retriever, labrador retriever, boxer, and terrier, have also been described as having an increased risk of developing cL (Flood-Knapik et al., 2013; Martini et al., 2016; Mizutani et al., 2016a; Seelig et al., 2014; Valli et al., 2006).

The occurrence of B-cell SLL is found in all breeds of dogs (Valli, 2007). Small breeds may be related with intestinal small cell lymphoma (SCL) of T-cell type. On the other hand, male gender can also be associated with intestinal small cell lymphoma (Couto et al., 2018; Lane et al., 2018).

3. Indolent Lymphoma Subtypes

The WHO has established a Lymphoma classification system, which is currently used for cL, based on the Revised European American Lymphoma (REAL) classification system. This system, created in 1994 and updated in 2000, classifies lymphomas based on phenotype, cell morphology, tissue architecture, location and their clinical course, differentiating them into aggressive or iL (Valli et al., 2011). According to the WHO classification, the following subtypes of iL are defined: marginal zone lymphoma, follicular lymphoma, mantle cell lymphoma, T-cell rich B-cell lymphoma, T-zone lymphoma, small lymphocytic lymphoma, lymphoplasmacytic lymphoma and intestinal small cell lymphoma (Flood-Knapik et al., 2013; Valli et al., 2017).

The canine indolent lymphoma (ciL) can originate from the proliferation of B or T-cells, and it can manifest as symmetric proliferations surrounding germinal centres or proliferations in peripheralized and fading germinal centres (Valli et al., 2011). This type of lymphomas commonly develops after an extended phase of benign hyperplasia, and in the initial phase, the iL may display medullary and capsular sclerosis. However, in advanced stages, the iL losses the follicle-related architecture holding identic cytomorphologic characteristics (Valli et al., 2006). As these authors pointed out, the absence of architecture can make it difficult to identify the indolent nature of the lymphoma on histological analysis.

Relating to incidence the studies have been shown:

i. some heterogenicity regarding the incidence of iL subtypes among studies. MZL was considered the most common subtype of ciL (Valli, 2007), with an incidence of 15-17% in one study (Valli et al., 2011). However, more recent studies support TZL as the most common subtypes of iL in dogs, with an incidence of 51.9% according to Flood-Knapik et al. (2013), and Seelig et al. (2016) and Parachini-Winter et al. (2018), mentioned that the incidence of TZL in dogs ranges between 15%-62%, accounting for 3% to 13% of all cL cases;
ii. a scarcity of MCL, FL, LPL and TCRBCL subtypes were reported in dogs (Flood-Knapik et al., 2013; Ponce et al., 2010; Valli, 2007; Valli et al., 2011).

Regarding to clinical presentation, and despite being characterized as a slowly progressive disease (Flood-Knapik et al., 2013; Parachini-Winter et al., 2018), the literature describes dogs with iL exhibit clinical heterogeneity. Some dogs show chronic but non-progressive clinical development, while others have a brief ST, and some may experience an evolution of the disease from indolent to aggressive (Aresu et al., 2015). According to this study, the mentioned heterogeneity implies a demanding management of treatment and clinical course. Although the transformation of iL to more aggressive histological subtypes is still poorly studied in veterinary oncology, there is evidence supporting its occurrence (Comazzi et al., 2015). The clinical presentation according to each iL subtype is resumed on table 1.

Table 1. Characterization of the subtypes of iL.

WHO Subtype (IPT)	Location	Cytology	Histology	Clinical signs	References
Marginal zone lymphoma (B)	- spleen; - nodal; - salivary glands; - intestine; - thyroid.	- nuclei of intermediate size with protuberant sole central nucleoli; - copious lightly stained cytoplasm; - mitoses absent except in advanced cases.	- indolent clonal proliferations of B lymphocytes of distinguishing architecture in which aggregates of neoplastic cells encircle fading fragments of germinal centres and look like the marginal zone of the lymph node follicle.	- maintains the appetite and action; - increases submandibular or cervical lymph node chain; - global lymphadenopathy.	Valli (2007) Valli et al. (2011) Seelig et al. (2016) Valli et al. (2017) Cozzi et al. (2018) Shiga et al. (2020)
Follicular lymphoma (B)	- lymph node; - spleen; - liver.	- mostly small cells with clear cytoplasm, light chromatin, and discreet nucleoli (centrocytes) with less large cells with dark blue cytoplasm, vesicular nuclei, and 1–3 nucleoli (centroblasts).	- germinal centre polarization in two zones: - apical light zone: composed of small-to-medium sized cells with relatively abundant amount of pale-staining cytoplasm; - deeper dark zone: with large cells that contain a narrow rim of dark-staining cytoplasm.	- lymph node enlargement is mild to moderate.	Valli (2007) Valli et al. (2011) Seelig et al. (2016)

WHO Subtype (IPT)	Location	Cytology	Histology	Clinical signs	References
Mantle cell lymphoma (B)	- lymph nodes; - spleen.	- small to intermediate-sized cells; - slight cytoplasm; - circular nuclei with dense chromatin: - unnoticeable nucleoli; - diverse mitotic rate.	- existence of fading germinal centres in some foci; - presence of small mature cells of the benign fading mantle cell cuff in the centre of some foci.	- generalized lymphadenopathy: - splenomegaly.	Valli (2007) Seelig et al. (2016)
T-zone lymphoma (T)	- peripheral lymph nodes	- small to medium-sized cells; -moderate quantity of lightly stained cytoplasm; - oval to elliptic nuclei with sharp shallow indentations; - finely granular chromatin; - inapparent nucleoli.	- areas of paracortex and medullary cords are filled with a uniform population of small or intermediate-sized lymphocytes; - prolonged pale cytoplasm - nuclei no internal nuclear particular.	- normal activity and appetite; - increase the peripheral lymph nodes, namely submandibular; - leukaemia may occur in advanced cases.	Valli et al. (2006) Valli (2007) Valli et al. (2011) Seelig et al. (2016)
Small cell lympho--cytic lymphoma (B or T)	- spleen; - liver - bone marrow - intestine - occasionally other tissues	- nuclei are only a bit larger than the red blood cells; - SLLs normally lack nucleoli and have a thin cover of basophilic cytoplasm - the cytologic diagnosis of SLL can be difficult as the cells are mature;	- nodal architecture: a population of uniformly small lymphocytes with neoplastic nodes; - diffuse cortical expansion of neoplastic cells compress the peripheral sinus and medullary cord regions; - tumor shapes compact sheets that appear compactly stained as the nuclei are small and near each other.	- asymptomatic; - leukemic state.	Valli (2007) Valli et al. (2017)

WHO Subtype (IPT)	Location	Cytology	Histology	Clinical signs	References
Lympho--plasma--cytic lymphoma (B)	- spleen - kidney - intestines - lymph nodes	- cells with intermediate-size and mostly without nucleoli; - cells with relatively wide and eccentrically located amphophilic cytoplasm.	- tumor cells have an explicit plasma cell morphology; - nuclei are compactly stained and are eccentric with a superficial nuclear indentation; - cytoplasm stains strongly pink.	- macroglobulinemia - paraproteinemia - good condition - normal appetite	Valli (2007) Valli et al. (2017)
T-cell rich B-cell lymphoma (B)	- liver	- large number of lymphocytes, mainly intermediate-sized, with smaller numbers of small lymphocytes and intermediate lymphocytes; - lymphoglandular bodies and numerous bare nuclei from ruptured cells.	- liver: a substitution of the normal liver parenchyma with an expansile, composed of lymphocytes arranged in nodular structures with atypical follicular outlines; - lymph nodes: there was an extended cortex with a nodular pattern. Follicular structures with a central lighter-staining zone enclosed by a dark-staining zone that occasionally compacted against vasculature and connective tissue.	- normal appetite - alert	Rout et al. (2018)
Intestinal small cell lymphoma	- gastrointestinal tract	- No data available	- lymphocytic infiltrates within the intestinal villous lamina propria and epithelium but often more pronounced in the villous tips.	- weight loss; - vomiting and diarrhoea - hypoalbuminemia.	Swerdlow et al. (2016) Lane et al. (2018) Couto et al. (2018) Matsumoto et al. (2019)

Legend: WHO - World Health Organization; IPT- immunophenotype.

4. Diagnosis

The multi-techniques diagnosis appears as the most adequate procedure for achieving a specific diagnosis of iL in canines (Valli et al., 2011). A variety of techniques can be used for diagnosis, but a combination of techniques is recommended to achieve a more accurate diagnosis. Further research is needed to improve knowledge in the field of diagnostics, as this is crucial for successfully managing different subtype of iL (Bienzle & Vernau, 2011; Seelig et al., 2016).

4.1. Fine-needle aspiration cytology

The results of the fine needle aspiration cytology (FNAC) are often used to trigger a number of second level of studies (flow cytometry, immunocytochemistry, biopsy with immunohistochemistry, clonality assays, and cytogenetic analysis). However, the studies showed that this technique is not enough as a single diagnostic for certain subtypes of lymphoma, including nodular and small-cell variants (Seelig et al., 2016).

In the case of enlarged peripheral lymph node (Ln) (>80% of the cases of canine nodal lymphomas), FNAC may be appropriate to filter the provisional list of differentials which includes reactive hyperplasia, lymphadenitis, lymphoma, and metastatic disease, because this technique is low-cost, safety, simple, and minimally-invasive sampling. In this line, it was widely accepted as first-line lymphoma diagnostic approach (Seelig et al., 2016).

FNAC compared with the biopsy has some limitations, namely, difficult to measure tissue architecture and unobtainable the material for additional studies (eg, IHC). On the other hand, blood sampling and aspiration of superficial masses and peripheral lymph nodes are secure and easy techniques. The diagnoses through cytology could not be appropriate in case of diffuse lymphomas composed of small or intermediate cells, as they seem like benign lymphocytes. In the FL cases, it is not easy to diagnose by cytology, due the mix of benign and malignant cells (Burkhard & Bienzle, 2015).

4.2. Biopsy *vs* fine needle-biopsy

The recommendations for the diagnosis of canine iL were the 2008 REAL-WHO guidelines. For histologic samples, the characteristics are: i) mitotic activity; ii) tumor architecture; and iii) tumor cell morphology (i.e., shape, size, and nuclear/cytoplasmic features). Moreover, to determine a concluding diagnosis it is also important to combine these items with the immunophenotype data and clinical disease burden (Seelig et al., 2016).

According to WHO requirements, the histological algorithm requires tissue sections 3-5 µm thick, stained with haematoxylin and eosin, and is reputed to be the gold standard for the diagnosis and classification of lymphoma. Further research is needed to understand the diagnostic value of non-excisional Ln biopsy trials (Seelig et al., 2016).

Fine-needle biopsy (FNB) is commonly used as the first-line diagnostic approach (Bienzle & Vernau, 2011). These authors highlighted several advantages and disadvantages of this technique, including:

i) advantages: the ability to obtain samples suitable for cytopathologic interpretation; can be executed with manual or no restraint in most dogs; can be done without general anaesthesia as required in incisional and excisional surgical biopsy; the specimens are suitable for immunocytochemistry, flow cytometry, recognition of clonal antigen receptor gene rearrangement, and genetic examination, if sufficient material is collected;
ii) disadvantages: samples are not appropriate to differentiate follicular from small cell lymphoma cases; evaluating tissue architecture; and the absence of tissue blocks for additional studies.

4.3. Immunohistochemistry

The WHO recommends histopathological classification and immunohistochemistry (IHC) with CD79a, CD3, Ki67 and P-glycoprotein to diagnose ciL (Flood-Knapik et al., 2013). IHC allows a correct classification of the subtypes of lymphoma (see table 2) as it helps in the histopathological subtypes. The value of the neoplastic cell immunophenotype it is irrefutable to make a diagnosis of cL. The diagnosis can be done by IHC, which is considered the gold standard, but also by flow cytometry, immunocytochemistry, and lymphoid clonality assays (Seelig et al., 2016). Flood-Knapik et al. (2013) proved the importance of IHC when testified a reclassification of 20% of indolent lymphomas cases with this tool.

Moreover, IHC plays several roles as (Flood-Knapik et al., 2013):

i) helps to clarify the lymphoma architecture and to detail follicle-related legacy;
ii) could give additional confirmation of the histopathological analysis as the follicular architecture of iL can be missing with time;
iii) could differentiating cases of TZL or MZL;
iv) using Ki67 could facilitate the differentiation between late-stage MZL and early diffuse large B cell lymphoma histopathological.

Table 2. Immunophenotypic of subtypes of iL.

Subtype	Phenotype	References
MZL	$CD1^+$, $CD20^+$, $CD21^+$, $CD45^+$, $CD79a^+$, $CD18^{intermediate}$, MHC II^+	Seelig et al. (2016) Vail et al. (2020)
FL	$CD20^+$, $CD21^+$, $CD45^+$, $CD79a^+$, MHC II^+	
MCL	$CD20^+$, $CD21^+$, $CD45^+$, $CD79a^+$, MHC II^+	
TZL	$CD45^-$, $CD3^+$, $CD5^+$, $CD21^+$, $CD4^{+/-}$, $CD8^{+/-}$	
SLL	B-SLL: $CD20^+$, $CD21^+$, $CD79a^+$, $CD3^-$ T-SLL: $CD3^+$, $CD20^+$, $CD21^+$, $CD79a^+$,	Valli et al. (2017)
LPL	$CD20^+$, $CD79a^+$, $CD3^-$	Valli et al. (2017)
TCRBCL	$CD3^+$, PAX5+, $CD10^{+/-}$	Rout et al. (2018)
INTESTINAL SCL	$CD3^+$, GRB^+, $CD8^+$, $CD56^-$	Matsumoto et al. (2019)

4.4. Flow cytometry

One of the methods that has gradually gained popularity for first-line analysis in cL diagnosis is flow cytometry of fine needle aspirates. These authors It was elected as a routine diagnosis technique for determining the immunophenotype of discrete cells and reinforced that this technique can be useful in cases with ambiguous results, as markers such as CD21, CD22, and CD20 are only expressed by mature B cells (Riondato & Comazzi, 2021).

The studies showed the advantages of combining FNAC with flow cytometry immunophenotyping, specifically: can analyse many antigens in one cell; needs only a little volume of sample; can identify rare and phenotypically aberrant cells; can give quantitative outcomes (Seelig et al., 2016). According to these authors, through the detection of tumor-specific immunophenotypic signatures (atypical light scatter-size and antigen expression shapes) the flow cytometry identifies and subclassifies lymphoid neoplasms and is important as an instrument of diagnostic hematopathology.

Flow cytometry was mentioned as a relevant diagnostic technique, particularly for identifying tumour-specific immunophenotypic signatures (size of erratic light scatter and antigen pattern inconsistent with ordinary lymphoid cells) (Seelig et al., 2016). However, the previous authors highlighted the combination of flow cytometry and cytology was used to diagnose canine low-grade lymphoma or non-neoplastic reactive hyperplasia. Flow cytometry findings showed a 100% incidence of neoplasia due to high prevalence of antigenic aberrations in canine small clear cell lymphoma and the absence CD45 expression, which can be considered a marker of neoplasia performed (Martini et al., 2015). These authors said that multicolour flow cytometry is a valuable tool to verify neoplasia and reject reactive hyperplasia in cases where histopathology is not performed.

4.5. Polymerase chain reaction for antigen receptor gene rearrangement

The polymerase chain reaction for antigen receptor gene rearrangement (PARR) allows the detection of clonal receptor gene rearrangement and the identification of T-cell or B-cell clonality in dogs, namely, B cells suffer rearrangement of genes encoding the immunoglobulin receptor; and T cells have rearrangement of genes encoding the T-cell receptor (TCR) (Burkhard & Bienzle, 2015).

Burkhard and Bienzle (2015) emphasized that, since neoplastic lymphocytes are of clonal origin, there are no cell surface indicators that recognize clonal T-cell populations in veterinary species or in human medicine. Furthermore, these authors mentioned that the value of light chain expression for the diagnosis of B-cell clonal populations is limited for dogs with lymphoma unlike what exists in human medicine. One of the strengths of PARR is the ability to detect a clonal population in early lymphoma arising amid a reactive population. Although, this technique could present false-positive and false-negative results. It worst to be mentioned that lymphoid clonality does not always relate to neoplasia.

4.6. Diagnostic features for each iL subtype

4.6.1 Marginal zone lymphoma

The diagnosis of MZL can be complex because this subtype of iL cannot be diagnosed by cytology (it is difficult to differentiate this subtype of lymphoma from marginal zone hyperplasia) and a definitive diagnosis requires architectural evaluation followed by immuno-phenotyping and molecular clonality assessment (O'Brien et al., 2013). Moreover, some cases of MZL may not be initially suspected based on the initial tests, as seen in a case study in which the outstanding features of the case were are not typical for MZL, namely: peripheral blood (PB) involvement; leukocytes in the blood with a monocytoid appearance; and a monoclonal gammopathy (Burgess et al., 2020). As these authors highlighted, gammopathies related with canine MZL have not been found in the literature.

The diagnosis of splenic MZL (sMZL) is not easy to perform (Stefanello et al., 2011), and several authors have recommended different multi-techniques for identification and diagnosis, namely:

- Valli et al. (2006) and Stefanello et al. (2011) recommend a combination of cytology, immunophenotype and molecular clonality assessment;
- Cozzi et al. (2018) suggests a cytology, histopathology and flow cytometry approach. The authors underlined that these techniques provided consistent information since these tumours have frequently similar cytologic features (small to medium-sized cells with a large central nucleolus), a low MI, and CD79a immunohistochemical positivity confirming a B-cell origin;
- Sabattini et al. (2018) proposed a histolopathology (considered the most valuable choice), immunophenotyping, and PARR can support the diagnosis of initial lesions, and the estimation

of proliferative activity with Ki-67 IHC can also contribute to the distinction between hyperplastic and neoplastic forms.

It is important to be mentioned the value of Ki-67 as a proliferation marker for categorizing cL into high and low-grade, such as in sMZL diagnosis (Riondato & Comazzi, 2021). However, it is not possible to differentiate large-sized high-Ki67 sMZL from splenic diffuse large B- cell lymphoma just by flow cytometry (Riondato & Comazzi, 2021). Moreover, sMZL commonly expresses CD5 in dogs (Stein et al., 2019).

4.6.2. Follicular lymphoma

There is a scarcity of knowledge regarding FL because it is a rare subtype of iL (Flood-Knapik et al. 2013), or this could be due to canine FL being just under-reported (Thomas et al., 2017).

Two cases of FL canine cutaneous lymphoma were reported, in which, IHC and flow cytometry analyses were performed, and the results showed that the majority of CD20-positive centrocytes in the follicles were strongly immunopositive for CD25, and high rates of CD25-positive cells were observed in the Ln samples (Mizutani et al., 2016a).

A case of colorectal FL was also reported (Richardson et al., 2019), and the authors advocated for the use of a combination histomorphology, clinical presentation, immunophenotyping and PARR testing to differentiate colorectal FL from unusual lymphoid hyperplasia. The results also suggested the use of a set of immunohistochemical markers (CD3, CD20, BCL2, BCL6) for the diagnosis of this disease. Moreover, the previous authors highlighted that the discrimination between colorectal FL and atypical lymphoid hyperplasia or other forms of iL could be important because colorectal FL cases have the potential to transform into an aggressive phenotype over time (Richardson et al., 2019).

4.6.3. Mantle cell lymphoma

A combined diagnosis with immunophenotype and histopathology is necessary for canine MCL and sMZL. By using histomorphological patterns along with a panel comprising BCL-2, BCL-6, MUM-1, and MCL-1 the diagnosis of canine MCL and MZL can be performed. Canine splenic MCL and MZL have distinct immunophenotypes and the key differences in between MCL and MZL are their immunoreactivity patterns for MUM-1 and MCL-1 However, further research is needed to corroborate these findings. Moreover, canine splenic MZLs generally express CD5 and consistent expression of BCL-2 with the lack of MCL-1 expression in MCL suggest potential therapeutic use of BCL-2 inhibitors, as used in human MCL, pending additional validation studies (Stein et al., 2019).

4.6.4. T-zone lymphoma

Dogs with TZL often show no signs of the disease during its development, which could be the reason of a later-stage diagnosis (Valli et al., 2013).

In the initial phase, the IHC is a valuable diagnostic technique for TZL diagnosis (as described in table 2) (Valli et al., 2011). However, over time, and according to some studies, flow cytometry has emerged as the most accurate technique for diagnosing TZL disease. Several reasons justify the use of flow cytometry, such as (Harris et al., 2019; Martini et al., 2015, 2016, 2017; Seelig et al., 2014;):

i) provides the complete immunophenotypic profile, and identifies typical CD45-negative TZL neoplastic cells (Seelig et al., 2014);
ii) recognizes unusual phenotype with variable T-cell markers and loss of expression for CD45 (Martini et al., 2015, 2016, 2017);
iii) is a non-invasive diagnostic technique used to classify the different subtypes of lymphoma by assessing cell size, cell complexity, and expression of different leukocyte antigens (Harris et al., 2019).

Nonetheless, other researchers suggest that flow cytometry should be combined with other techniques to achieve a broaden diagnosis of TZL (see figure 1) (Mizutani et al., 2016a; Parachini-Winter et al., 2018; Rout & Avery, 2017).

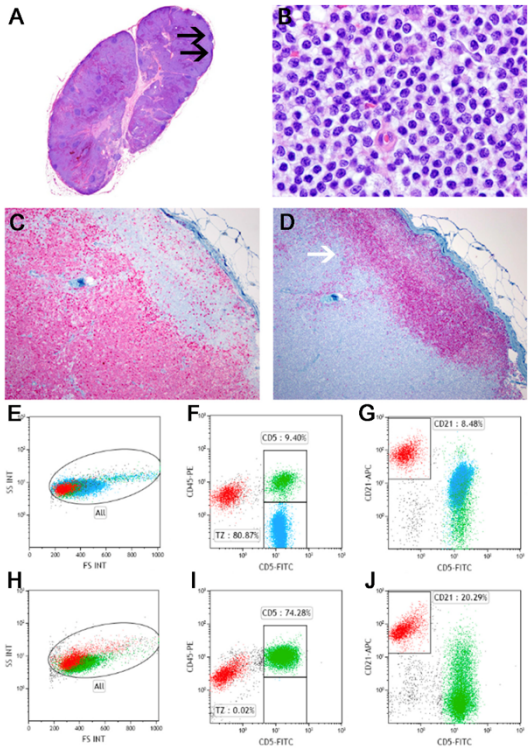

Figure 1. The histologic, immunohistochemical and flow cytometry appearance of canine T-zone lymphoma (Seelig et al., 2016:14).

There are recommendations that PARR could be more precise than flow cytometry when the clonal cells are a minority of the lymphocyte population. Mizutani et al. (2016a) recommended that TZL diagnosis should involve several techniques, specifically cytology, histopathological examination and PARR (although the sensitivity for TCRγ genes may be low). Moreover, the previous authors proposed that PARR could be combined as part of the diagnostic protocol to detect a preneoplastic state that may not be detectable with flow cytometry or other diagnostic techniques, especially in the golden retriever breed, which has a higher incidence of TZL (Hughes et al., 2018). Additionally, fine-needle aspiration cytology samples can be used for immunophenotyping, either by immunocytochemistry and flow cytometry, allowing for a less invasive and more rapid diagnostic approach (Seelig et al., 2014).

A new type of TZL in the tongue was reported in a case series of twelve dogs (Harris et al., 2018). This study demonstrated $CD3^+$ T-cell origin and loss of CD45 expression, both by flow cytometry; the presence of small number of PAX5 and CD79-alfa positive B-cells in histologic samples; infiltration of the mucosa by neoplastic cells linked to lymphoid tissue; and increased expression of CD21 in neoplastic cells.

4.6.5. Small lymphocytic lymphoma

The diagnosis of SLL with cytology can be difficult because the lymphocytes are mature, though the diagnosis may be performed through flow cytometry, IHC or PARR (Valli, 2007; Valli et al., 2017).

However, it is important to note that this tumour has the potential to progress to a large cell lymphoma or undergo pro-lymphocytic or blastic transformation with or without leukemic involvement, often accompanied by hepatosplenomegaly (Valli, 2007).

The diagnostic features taken to predict the progression of CLL a in veterinary medicine are: relatively normal blood leukocyte analysis, minor to moderate anemia, scarce mitoses in aspirated cells of liver and spleen, and phenotypic staining for B-cell type (Valli, 2007).

4.6.6. Lymphoplasmacytic lymphoma

Lymphoplasmacytic lymphoma could be a cellular proliferation of B-cell type, characterized by a surface and usually cytoplasmic IgM and stain positive for CD79 alpha and CD20 (Valli, 2007). Its diagnosis is based on the recognition of nodal, splenic, and bone marrow (BM) proliferation, but all tissues affected by focal LPL cell invasion (in general the exceptions are the central nervous system, kidney and lung) (Valli, 2007). In some cases, there may be the presence of high molecular weight serum paraprotein. Notably, LPL of T-cell is more frequently observed in the small intestine (5/6, 83%) compared to the large intestine (1/6, 17%) (Kojima et al., 2021).

4.6.7. T-cell rich B-cell lymphoma

In a case report of T-cell rich B-cell lymphoma, the diagnosis was achieved by combining IHC, flow cytometry and PARR (Rout et al., 2018). The IHC, revealed marked atypia of the follicular structures and a diffuse cytoplasmic staining for CD3. The surrounding small lymphocytes in the dark-staining zones had strong diffuse nuclear staining for PAX5 (Rout et al., 2018). The flow cytometry, revealed a mixed population with $CD4^+$ T cells and $CD8^+$ T cells (small cells), about one-third of $CD3^+$ $CD5^+$ T cells expressed low levels of CD21. A minor population of small $CD21^+$ $CD5^-$ B cells, accounting for approximately 5% of the total cell population, was also observed. Additionally, in this case report, around 15% of the population consisted of small leukocytes stating only CD45 (pan-leukocyte marker) and MHC II. The PARR technique, showed clonal immunoglobulin and polyclonal T-cell receptor rearrangements (Rout et al., 2018).

4.6.8. Intestinal small cell lymphoma

Diagnosis of intestinal small cell lymphoma in dogs was primarily established using IHC (Lane et al., 2018). However, in some cases, the diagnosis was further confirmed through the identification of a monoclonal neoplastic cell population on PARR analysis (Lane et al., 2018). It is important to be mentioned that IHC, PARR or both are frequently required to distinguish lymphocytic inflammation from low-grade lymphoma or chronic enteropathy in dogs (Carrasco et al., 2015; Nakashima et al., 2015).

In a study developed by Couto et al. (2018), the diagnosis of intestinal small cell lymphoma was performed for all cases through IHC and the polymerase chain reaction (PCR) to show T-cell morphology. A clonal rearrangement of TRG was confirmed in 15 (88%) dogs. These authors emphasized the importance of performing histopathology, IHC and PCR in all cases where a lymphocytic infiltrate is present in the intestinal tract to accurately identify cases that truly represent small cell intestinal lymphoma.

5. Staging

Staging is a critical and ongoing process in cancer management that helps to determine the most appropriate strategy for approaching the disease (Vail et al., 2020).

The WHO has developed a classification system for clinical staging of lymphoma in domestic animals, which is also used for iL. This system defines five main stages and two substages were defined (table 3) (Vail et al., 2020). According to this classification, stages I and II are associated with a local disease, whereas stage V indicates that the disease has become systemic. In the case of the primary splenic lymphoma the WHO classification can lead to some misunderstanding, as it is characterized as a subtype with a more indolent course compared to other stage IV tumours (van Stee et al., 2015).

Table 3. WHO clinical staging system for lymphoma in domestic animals (adapted from Vail et al., 2020: 693).

Stage	
I.	Involvement limited to a single node or lymphoid tissue in a single organ[a]
II.	Involvement of many lymph nodes in a regional area (±tonsils)
III.	Generalized lymph node involvement
IV.	Liver and/or spleen involvement (± Stage III)
V.	Manifestation in the blood and involvement of bone marrow and/or other organ systems (± Stage I-IV)
Substage	
a.	Without systemic signs
b.	With systemic signs

Legend: [a] Excluding bone marrow.

The stage of the lymphoma is related to a median survival time (MST), and consequently with different prognosis; dogs in stage 1, 2 and 3 with MST of 324, 218, and 199 days, respectively (Valli et al., 2013).

The European Canine Lymphoma Network concluded that staging methods varied among studies (Marconato et al., 2017), with the most used staging practices including complete blood cell counts (100%), serum biochemical profile (83,1%) and/or urinalysis (100%), thoracic radiographs (82,7%), BM evaluation (73,1%), abdominal ultrasound examination (59,6%) and abdominal radiographs (28,8%). PB and BM assessment are crucial for defining the stage of cL, and flow cytometry is recommended (Marconato, et al., 2019a; Riondato & Comazzi, 2021). This evaluation provides data that can be associated with first remission and survival (Marconato et al., 2019a).

Findings of several studies have shown that dogs with indolent T-cell lymphoma with stage V disease had clinical signs at diagnosis (Aresu et al., 2015; Flood-Knapik et al., 2013; Valli et al., 2006). Aresu et al. (2015) found that most of the indolent B-cell lymphoma was diagnosed in clinical stages IV and V with a high percentage (38.1%) of dogs with clinical stage V disease.

In advanced stages, certain organs were preferentially involved, specifically the liver, spleen, and BM (Aresu et al., 2015). As these authors stated, the liver is more commonly involved in aggressive B-cell and indolent T-cell lymphomas than in aggressive T-cell lymphomas. The spleen is more often involved in aggressive B-cell lymphomas than in indolent T-cell lymphomas, and in indolent T-cell

lymphomas than in aggressive T-cell lymphomas. BM infiltration is more frequent in all T-cell lymphomas than in aggressive B-cell lymphomas (Aresu et al., 2015).

6. Treatment and survival

The appropriate treatment for lymphoma depends on an accurate diagnosis and a deep understanding of the histological subtypes (Aresu et al., 2015). However, there is still debate regarding the systemic treatment for iL, as studies have shown that longer ST can be achieved without any treatment (Burgess et al., 2020; Flood-Knapik et al., 2013).

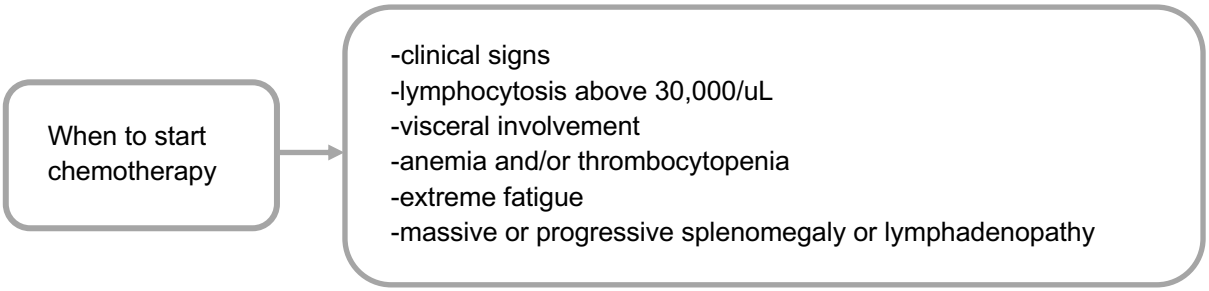

Figure 2. The trigger to start chemotherapy in iL (adapted from Lorimier and Campbell (2020), and Mizutani et al., 2016a).

6.1. Chemotherapy

Chemotherapy for canine iL treatment comprises several treatment protocols such as: only prednisone; chlorambucil and prednisone; cyclophosphamide-hydroxydaunorubicin-vincristine and prednisolone (CHOP protocol) (Burgess et al., 2020). As previous mentioned, the use of chemotherapy is controversial, but some authors recommend its use in several situations, resumed on figure 2. A conservative oral protocol such as prednisone and chlorambucil has shown good responses in dogs with iL and favourable long- term prognosis (Valli et al., 2006; Flood-Knapik et al., 2013; O'Brien et al., 2013; Seelig et al., 2014; Stefanello et al., 2011; Thamm, 2019). Systemic chemotherapy can be a viable option for dogs with iL with advanced disease or a clinical course similar to high-grade lymphomas (Valli et al., 2006). However, Flood-Knapik et al. (2013) reported that the use of systemic chemotherapy did not influence overall survival in dogs with MZL and TZL. Moreover, the factor that had a greater influence on ST was the magnitude of lymphocytosis, rather than the chemotherapy applied (Flood-Knapik et al., 2013).

Table 4. Studies on the treatment and survival of TZL.

Authors	Treatment	Median survival (days)
Valli et al. (2006)	CHOP protocol	686
	Prednisone	686
Valli et al. (2013)	Any hydroxydaunorubicin-containing protocol	409
	Any non-hydroxydaunorubicin-containing protocol	314
	No treatment	689
Flood-Knapik et al. (2013)	CHOP protocol	470
	Chlorambucil and prednisone	median was not reached, (>50 per cent alive)
Mizutani et al. (2016b)	CHOP protocol	938
	Melphalan and prednisolone	
	Prednisolone	
	No treatment	

Dogs with TZL may have a double approach (Valli et al., 2013). Some dogs may require therapy based on the specific phase of the disease, while others can live for years without treatment. Furthermore, in cases where chemotherapy is necessary, the melphalan and prednisolone protocol has shown to be a successful procedure with fewer side effects. The watchful waiting approach appears to be a suitable option for dogs without any other issues and blood cytopenias (Mizutani et al., 2016b). Moore (2016: 279) recommended that the initiation of the protocol should consider the following criteria: “clinical signs (substage b); rapid progression (doubling time of <6 months); circulating lymphocyte count >9.2; bulky multiple sites >3 cm or one site >7 cm; development of myelosuppression due to myelophthisis; or organ dysfunction due to infiltration”. This author also emphasizes the importance of careful monitoring of haematology and peripheral Ln before starting chemotherapy.

The findings of the study by Aresu et al. (2015) revealed interesting results. Dogs with indolent or aggressive T-cell lymphoma, treated with the same chemotherapy, had the same median survival (55 days). On the other hand, dogs with indolent B-cell lymphoma or aggressive B-cell, subjected to the same treatment, comprising a more aggressive therapeutic intervention (CHOP-based multidrug chemotherapy; L-asparaginase, vincristine, cyclophosphamide, doxorubicin, lomustine and prednisone), generally had a longer median survival compared to T-cell cL, with 200 and 256 days respectively.

A group of dogs with nodal MZL (nMZL) in an advanced stage of the disease was treated with CHOP-based protocol, however the time to progression (TTP) and lymphoma specific survival (LSS)

was variable according to the prognosis factors (Cozzi et al., 2018). In this study dogs with increased lactate dehydrogenase and substage b) had less LSS days compared with dogs with normal lactate dehydrogenase and substage a), respectively 211 days and 125 days. These authors hypothesized that this could be due to the relatively low dose intensity and suggested that the incorporation of different alkylating drugs may better target MZL cells.

There are few studies focusing on the treatment and survival focusing on the low-grade GI lymphoma in dogs. However, the study developed by Lane et al. (2018), which included 20 cases, showed that the chemotherapy approach had a good response, with a satisfactory MST of 535 days. Dogs with intestinal SCL appear to have a favourable outcome regardless of the type of therapy, with an MST of 628 days (Couto et al., 2018). A combination of chlorambucil and prednisone was reported with a MST of 424 days (Rassnick et al., 2009). Previous reports have indicated that dogs with protein losing enteropathies, where a clonal population was demonstrated, had a worse overall prognosis compared to dogs with a polyclonal result (Maeda et al., 2017; Ohmi et al., 2017). It is uncertain whether this indicates true transformation of the small cell intestinal lymphoma or de-novo high-grade lymphoma due to the absence of validation with matched molecular clonality assessment. Further research focusing on this subtype is needed to determine the existence of true conversion to high-grade lymphoma.

6.2. Chemo-Immunotherapy

One strategy for enhancing chemotherapy in dogs with advanced indolent B-cell lymphoma was vaccination with an autologous vaccine consisting of hydroxyapatite ceramic powder and heat shock proteins purified from the dogs tumours (HSPPCs-HA) (Marconato et al., 2015). Chemo-immunotherapy appears to be a more successful approach compared to treatment with chemotherapy alone (Marconato et al., 2019a; Marconato et al., 2014). In cases of dogs with with nMZL treated with chemo-immunotherapy, TTP and LSS were significantly longer compared with dogs treated with chemotherapy only (consisting of L-Asparaginase, Vincristine, Cyclophosphamide, Doxorubicin, Lomustine, and Prednisone) (Marconato et al., 2019a).

Similarly, in dogs with indolent B-cell lymphoma it was found that those receiving chemo-immunotherapy had a significant improvement in TTP compared to dogs receiving chemotherapy alone and the LSS tended to be longer (Marconato et al., 2015). This approach produced clinical benefits with no increased toxicity, resulting in significantly longer TTP, but LSS only tended to be longer (Marconato et al., 2015). The findings of the study by Marconato et al. (2019b) demonstrated that dogs with FL had higher survival rates, in the chemo-immunotherapy group than in chemotherapy group. Dogs with nMZL had higher survival rates, for 1-year and for 2-year, in the chemo-immunotherapy group compared with in the chemotherapy group, but for 3 years the survival rate was the same in both groups (10%) (Marconato et al., 2019b). Nonetheless, further research is needed to better understand this

immunotherapy approach and to develop a deeper knowledge regarding vaccination protocol (doses and frequency), the duration of responses, and the ideal timing for booster vaccinations (vaccination should be performed after the induction phase of chemotherapy with dogs in complete remission) (Marconato et al., 2015).

6.3. Surgery

In case of the splenic B-cell lymphoma that is restricted to the spleen, splenectomy and monitoring alone appear to be a successful approach, with a 1-year survival rate in 69,8% of the cases, moreover, the treatment with chemotherapy did not increase the ST (van Stee et al., 2015). The reports of Valli (2007) and Flood-Knapik et al. (2013) also confirmed these findings, namely and respectively, dogs treated only with splenectomy, none of them died from lymphoma during the follow-up period; dogs undergoing primary splenic lymphoma surgery had a LSS of 21.6 months.

For sMZL, splenectomy seems to be a successful approach (Zandvliet, 2016). However, a combined approach with splenectomy and adjuvant chemotherapy has shown longer survival compared to those undergoing surgery alone and it was suggested that this combined approach may be important in eliminating minimal residual disease (Stefanello et al., 2011). On the other hand, good results were found with splenectomy alone, which considered the treatment of choice in dogs with sMZL. However, adjuvant chemotherapy (cytotoxic injectable chemotherapy) may not prolong ST in dogs with MZL (O'Brien et al., 2013).

Dogs with colorectal FL that were submitted to surgery had a lengthy ST (Richardson et al., 2019). In this study, one of the dogs diagnosed with colorectal FL in this series developed multicentric lymphoma following resection of 1 of the 2 reported colorectal masses. The treatment was cyclophosphamide, hydroxydaunorubicin, vincristine and prednisone (CHOP). The dog responded well to therapy and has remained in remission for at least 2 years (Richardson et al., 2019).

7. Final Remarks

The incidence of iL has been increasing; however, the aetiology of iL is still scarcely studied, although it is known that there are some predisposing factors, such as breed. There is no established relationship between the incidence of iL and age, but several studies have concluded that it mainly affects older animals, particularly in TZL and MZL, while FL seems to affect younger dogs. The irregular geographical prevalence among lymphoma subtypes could be explained by different environmental risk factors in each area, both within and between continents.

Over the years, with the increasing number of studies, the incidence of different iL subtypes has been changing. The most common subtype was reported to be MZL, but recent studies indicate that TZL is now the most common subtype among iL cases.

The clinical signs of iL are non-specific, and dogs can maintain their activity and appetite until advanced stages. However, early detection of these disease subtypes is crucial before a potential transformation into a more aggressive grade.

Research in this area has gained interest in recent years, particularly in terms of new diagnostic techniques and treatments. A multidisciplinary approach is necessary for accurate diagnosis and appropriate therapeutic management. Immunophenotypic characterization of lymphoid neoplasms, obtained through FNB samples and analysed using flow cytometry, is a valuable tool in veterinary oncology.

As research points out, therapeutic approaches should be tailored to the histological subtype of iL, as there is no one-size-fits-all therapeutic approach. The goal of therapy is disease control (stable disease or partial response), since complete remission is uncommon in iL. Chemotherapy, immunochemotherapy and surgery have different recommendations depending on the iL subtype.

Dogs with indolent low-grade lymphomas have a poorer response to chemotherapy, but still have a survival advantage compared to dogs with intermediate and high-grade lymphomas. In addition, research has shown that dogs with iL have prolonged ST, often just watchful and waiting.

The recommendation for iL include active surveillance in the absence of treatment, more conservative protocols (chlorambucil/prednisone or cyclophosphamide/prednisone) in cases where a therapeutic approach is available. In sMZL, treatment with splenectomy appears to be a successful approach. Several case compilations have documented that dogs with indolent lymphomas have prolonged STs, often in the absence of any treatment or aggressive chemotherapy. Many dogs with indolent lymphoma can live near-normal life spans and eventually die of diseases unrelated to the lymphoma.

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