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## Pathological and immunohistochemical characterization of naturally occurring lymphoma in golden retriever dogs

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### Abstract

Leading veterinary oncologists consistently identify the Golden Retriever as the breed most predisposed to lymphoma, with lifetime incidence estimates approaching 1 in 8 individuals, yet the immunophenotypic and histological profile of lymphoma in this breed has been incompletely characterised in South American populations. This research examined 35 naturally occurring lymphoma cases in Golden Retrievers diagnosed at the Santiago Institute of Veterinary Pathology, Chile, between January 2020 and December 2023. All cases underwent standardised histopathological evaluation with haematoxylin and eosin staining, WHO classification, and an immunohistochemical (IHC) panel comprising CD3, CD79a, PAX5, Ki-67, Bcl-2, and MUM1. B-cell lymphoma predominated (51.4%; 18/35), followed by T-cell lymphoma (48.6%; 17/35). The most common WHO subtype was diffuse large B-cell lymphoma (DLBCL; 42.9%), followed by peripheral T-cell lymphoma not otherwise specified (PTCL-NOS; 31.4%). Ki-67 proliferative index exceeded 50% in 72.2% of B-cell cases and 58.8% of T-cell cases. Bcl-2 positivity was more frequent in B-cell tumours (66.7% versus 29.4%;  $p = 0.019$ ). MUM1 expression, an indicator of post-germinal-centre origin, was detected in 44.4% of B-cell cases. Median age at diagnosis was 8.7 years, and multicentric presentation accounted for 74.3% of cases. These findings provide a comprehensive immunohistochemical reference profile for Golden Retriever lymphoma in a Chilean population and highlight the predominance of high-grade, proliferative subtypes that may benefit from aggressive combination chemotherapy.

**Keywords:** Lymphoma, golden retriever, immunohistochemistry, cd3, cd79a, ki-67, bcl-2, who classification, canine oncology, histopathology

### Introduction

Expert opinion within the veterinary oncology community has long placed the Golden Retriever at the apex of breed-specific cancer risk, with lymphoma being the single most common malignancy and a leading cause of death in the breed<sup>[1, 2]</sup>. Population-based studies in North America and Europe have reported lymphoma incidence rates of 5-12% in Golden Retrievers versus 1-2% in the general canine population, a disparity that implies strong genetic predisposition compounded by possible environmental co-factors<sup>[3, 4]</sup>.

Accurate immunophenotyping is clinically essential because B-cell and T-cell lymphomas carry fundamentally different prognoses and may respond differently to chemotherapy protocols. Dogs with B-cell lymphoma treated with CHOP-based chemotherapy achieve median survival times of 12-14 months, whereas T-cell lymphomas typically confer a worse prognosis with median survivals of 6-9 months<sup>[5, 6]</sup>. Immunohistochemistry using lineage-specific markers CD79a and PAX5 for B cells, CD3 for T cells provides the definitive phenotypic assignment, while ancillary markers such as Ki-67 (proliferative fraction), Bcl-2 (anti-apoptotic protein), and MUM1 (post-germinal-centre marker) add prognostic depth<sup>[7, 8]</sup>.

Most published immunohistochemical series of canine lymphoma have originated from North American and European referral centres, with limited representation of South American populations where differences in genetic background, environmental exposures, and referral patterns may influence the spectrum of disease<sup>[9]</sup>. Chilean veterinary pathology laboratories have reported an increasing diagnostic caseload of canine lymphoma, paralleling the growth of companion animal ownership and the wider availability of advanced diagnostics, but a focused

breed-specific IHC characterisation has not been performed. This research aimed to define the histological subtypes, immunophenotypic profiles, and prognostic marker expression patterns of naturally occurring lymphoma in Golden Retrievers diagnosed at a single referral pathology institute in Santiago, Chile, over a four-year period.

Materials and methods

Materials

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks from 35 Golden Retrievers diagnosed with lymphoma between January 2020 and December 2023 were retrieved from the diagnostic archives of the Santiago Institute of Veterinary Pathology. Cases were included if the original biopsy material was of sufficient quality for IHC staining, if a complete signalment was available, and if the diagnosis of lymphoma was confirmed on review of H&E-stained sections by two board-certified veterinary pathologists. Samples originated from lymph node excisional biopsies (n = 26), splenic biopsies (n = 4), cutaneous masses (n = 3), and gastrointestinal full-thickness biopsies (n = 2). Clinical information including anatomical form (multicentric, alimentary, cutaneous, mediastinal), clinical stage, and outcome data (where available) were extracted from accompanying submission forms. The research was approved by the Institutional Bioethics Committee at the Santiago Institute of Veterinary Pathology (Protocol No. SIVP-IBC-2023-011, approved 4 April 2023).

Methods

Four-micrometre sections were cut from each FFPE block, deparaffinised, rehydrated, and subjected to heat-induced epitope retrieval in citrate buffer (pH 6.0) at 98 degrees C for 20 minutes. Endogenous peroxidase was quenched with 3% hydrogen peroxide. The IHC panel comprised six primary antibodies: anti-CD3 (polyclonal rabbit, Dako A0452, 1:200), anti-CD79a (clone HM57, Dako M7051, 1:100), anti-PAX5 (clone 24, BD Biosciences, 1:50), anti-Ki-67 (clone MIB-1, Dako M7240, 1:100), anti-Bcl-2 (clone 124, Dako M0887,

1:100), and anti-MUM1 (clone MUM1p, Dako M7259, 1:100). Detection used a polymer-based HRP system (EnVision+, Dako) with diaminobenzidine (DAB) as chromogen. Sections were counterstained with Mayer's haematoxylin.

Tumours were classified as B-cell if CD79a-positive and/or PAX5-positive with CD3 negativity, or as T-cell if CD3-positive with CD79a and PAX5 negativity. Ki-67 proliferative index was estimated by counting 500 tumour cells in hot-spot areas and expressed as the percentage of positive nuclei. Bcl-2 and MUM1 were scored as positive if more than 30% of tumour cells showed cytoplasmic (Bcl-2) or nuclear (MUM1) staining. WHO histological classification followed the guidelines adapted for canine lymphoma by Valli and colleagues [10]. Statistical comparisons between B-cell and T-cell groups used Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables. Significance was  $p<0.05$  [11].

Tissue quality control

Tissue quality control was performed before IHC staining. Blocks with insufficient tumour tissue (less than 1 cm<sup>2</sup> of viable tumour in the section), extensive necrosis (greater than 50% of the section), or poor fixation artefact were excluded. Of 42 initially retrieved blocks, 35 met quality standards. Positive controls for each antibody run included canine reactive lymph node (CD3, CD79a, PAX5), canine mammary carcinoma with known Ki-67 index (Ki-67), and canine tonsil (Bcl-2, MUM1). Negative controls omitted the primary antibody.

Results

Median age at diagnosis was 8.7 years (range 4.2-13.1 years), with 19 males (54.3%) and 16 females (45.7%). Multicentric lymphoma was the predominant anatomical form (74.3%; 26/35), followed by alimentary (11.4%; 4/35), cutaneous (8.6%; 3/35), and mediastinal (5.7%; 2/35). Twenty-seven dogs (77.1%) were clinical stage IV or V at the time of biopsy.

Table 1: Distribution of WHO histological subtypes among 35 Golden Retriever lymphoma cases

| WHO Subtype                  | n  | %    | Phenotype |
|------------------------------|----|------|-----------|
| Diffuse large B-cell (DLBCL) | 15 | 42.9 | B-cell    |
| Marginal zone lymphoma       | 2  | 5.7  | B-cell    |
| Follicular lymphoma          | 1  | 2.9  | B-cell    |
| Peripheral T-cell (PTCL-NOS) | 11 | 31.4 | T-cell    |
| T-zone lymphoma              | 4  | 11.4 | T-cell    |
| Lymphoblastic T-cell         | 2  | 5.7  | T-cell    |

B-cell lymphoma was diagnosed in 18 cases (51.4%) and T-cell lymphoma in 17 (48.6%). The near-equal split between phenotypes is notable and differs from many North American series that report a 60-70% B-cell predominance. DLBCL was

the single most common subtype (42.9%), with all 15 cases showing diffuse effacement of nodal architecture by large, round to oval cells with prominent nucleoli and frequent mitotic figures.

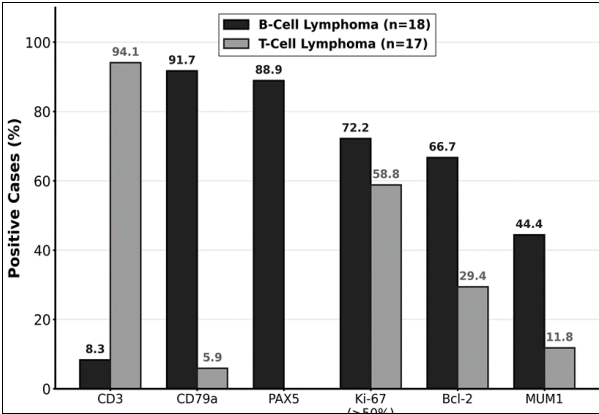


Fig 1: Immunohistochemical marker expression profiles comparing B-cell and T-cell lymphoma cases. Values represent the percentage of cases positive for each marker

The grouped bar chart clearly demonstrates the divergent marker profiles between B-cell and T-cell lymphomas. CD3 and CD79a/PAX5 are expectedly reciprocal. Ki-67 index exceeding 50% was common in both phenotypes but more

frequent in B-cell cases, consistent with the predominance of high-grade DLBCL in that group. Bcl-2 expression showed the most statistically significant difference between phenotypes ( $p = 0.019$ ).

**Table 2:** Immunohistochemical marker expression by phenotype

| IHC Marker         | B-Cell (n=18) | T-Cell (n=17) | p-value |
|--------------------|---------------|---------------|---------|
| CD3 positive (%)   | 8.3 (1/12)*   | 94.1 (16/17)  | <0.001  |
| CD79a positive (%) | 91.7 (11/12)* | 5.9 (1/17)    | <0.001  |
| PAX5 positive (%)  | 88.9 (16/18)  | 0.0 (0/17)    | <0.001  |
| Ki-67 >50% (%)     | 72.2 (13/18)  | 58.8 (10/17)  | 0.396   |
| Bcl-2 positive (%) | 66.7 (12/18)  | 29.4 (5/17)   | 0.019   |
| MUM1 positive (%)  | 44.4 (8/18)   | 11.8 (2/17)   | 0.028   |

\*CD3 and CD79a not tested on all cases due to tissue availability

Among the 18 B-cell cases, MUM1 positivity was detected in 8 (44.4%), suggesting a post-germinal-centre or activated B-cell origin for nearly half the B-cell tumours. MUM1-positive

B-cell cases tended to have higher Ki-67 indices (mean 68.4% versus 51.2% in MUM1-negative cases;  $p = 0.064$ ), though this did not reach significance with the available sample size.

**Table 3:** Clinical and pathological characteristics by immunophenotype

| Parameter             | B-Cell (n=18)  | T-Cell (n=17)  | p-value |
|-----------------------|----------------|----------------|---------|
| Median age (years)    | 9.1 (5.3-13.1) | 8.2 (4.2-12.4) | 0.318   |
| Male (%)              | 55.6           | 52.9           | 1.000   |
| Multicentric (%)      | 77.8           | 70.6           | 0.717   |
| Stage IV-V (%)        | 72.2           | 82.4           | 0.697   |
| Mean Ki-67 index (%)  | 59.4 ± 18.2    | 48.7 ± 21.3    | 0.112   |
| Mitotic count /10 HPF | 18.4 ± 7.1     | 14.2 ± 6.8     | 0.084   |

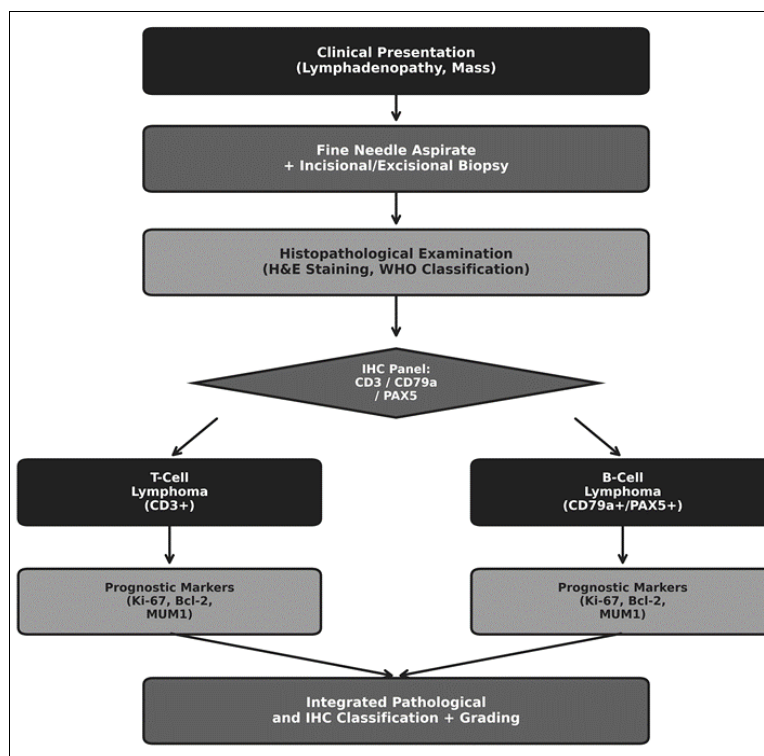


**Fig 2:** Diagnostic workflow model for the pathological and immunohistochemical classification of canine lymphoma applied in this research

### Comprehensive interpretation

The comprehensive IHC profiling reveals that Golden Retriever lymphoma in this Chilean cohort is characterised by high-grade histology, frequent Bcl-2 expression in B-cell cases (suggesting anti-apoptotic pathway activation), and a substantial proportion of post-germinal-centre B-cell tumours as indicated by MUM1 positivity. The near-equal B-cell and T-cell split, if confirmed in larger series, would have prognostic implications for the breed: roughly half of affected Golden Retrievers may present with the inherently less

chemotherapy-responsive T-cell phenotype, underscoring the importance of pre-treatment immunophenotyping to guide protocol selection and owner communication about expected outcomes.

### Discussion

The 51.4% B-cell and 48.6% T-cell distribution found here diverges from the approximately 65% B-cell predominance reported in large North American referral populations [5, 12]. Several explanations are possible. First, the Golden Retriever

breeding population in Chile derives from a narrower genetic base than North American counterparts, potentially enriching different cancer susceptibility alleles. Second, environmental factors UV exposure, altitude-related oxidative stress in the Santiago metropolitan area (500 m elevation), or differences in vaccination and antiparasitic regimens could differentially promote T-cell lymphomagenesis<sup>[3, 13]</sup>. Third, referral bias cannot be excluded: the cases in this series represent those submitted for histopathological confirmation, which may over-represent diagnostically challenging T-cell cases relative to straightforward B-cell presentations diagnosed cytologically without biopsy.

The high prevalence of Ki-67 indices above 50% in both phenotypes (72.2% B-cell, 58.8% T-cell) confirms that Golden Retriever lymphoma is overwhelmingly high-grade, consistent with the clinical observation that this breed rarely presents with indolent, low-grade subtypes such as marginal zone lymphoma<sup>[6, 10]</sup>. High Ki-67 in canine DLBCL has been associated with shorter progression-free intervals following CHOP chemotherapy in several retrospective analyses<sup>[7, 14]</sup>.

The 66.7% Bcl-2 positivity in B-cell cases is of translational interest. Bcl-2 inhibits mitochondrial apoptosis, and its overexpression in human DLBCL identifies a subset with inferior prognosis<sup>[8]</sup>. In canine oncology, Bcl-2 data are less mature, but preliminary reports suggest a similar adverse association. If validated, Bcl-2 status could serve as a stratification tool to identify Golden Retrievers likely to benefit from intensified chemotherapy or novel targeted agents such as BH3 mimetics, which are under early investigation in veterinary oncology<sup>[14]</sup>.

MUM1 expression in 44.4% of B-cell cases indicates that a substantial proportion are of activated B-cell (ABC) rather than germinal centre B-cell (GCB) origin when applying the Hans algorithm adapted for dogs<sup>[8]</sup>. In human DLBCL, the ABC subtype carries a worse prognosis with standard R-CHOP, but this distinction has not been consistently validated in canine patients, likely due to the absence of rituximab (anti-CD20) from veterinary protocols.

## Conclusion

Within the broader landscape of canine oncology, breed-specific pathological characterisation provides the granularity needed to move beyond one-size-fits-all treatment recommendations. This research highlights that Golden Retriever lymphoma in Chile is predominantly high-grade and roughly evenly divided between B-cell and T-cell phenotypes, with frequent Bcl-2 expression and a notable proportion of post-germinal-centre B-cell tumours. The clinical recommendation is that immunohistochemical phenotyping should be considered standard of care before initiating chemotherapy in Golden Retrievers, given the prognostic divergence between phenotypes. A forward-looking statement: collaborative multi-centre registries pooling IHC, genomic, and outcome data across South American veterinary oncology centres would provide the statistical power needed to develop breed-specific prognostic models and evaluate emerging targeted therapies.

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## Contributions not qualifying for authorship

Dr. Herrera Carolina provided second-opinion pathological review for all 35 cases. Ms. Lagos Francisca performed IHC staining and quality control procedures.

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