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Large granular lymphocyte lymphoma in 65 dogs (2005–2023)

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Abstract

Large granular lymphocyte lymphoma (LGLL) is a rare form of lymphoma in dogs. Limited information exists regarding presentation, treatment response, and outcome. The aim of this single-institute, retrospective study was to characterise clinical presentation, biologic behaviour, outcomes, and prognostic factors for dogs with LGLL. Cytologic review was also performed. Sixty-five dogs were included. The most common breed was the Labrador retriever (29.2%), and the most common presenting signs were lethargy (60.0%) and hyporexia (55.4%). The most common primary anatomic forms were hepatosplenic (32.8%) and gastrointestinal (20.7%). Twenty dogs (30.8%) had peripheral blood or bone marrow involvement. Thirty-two dogs were treated with maximum tolerated dose chemotherapy (MTDC) with a response documented in 74.1% of dogs. Dogs ≥7 years, and those with neutropenia or thrombocytopenia at diagnosis had the reduced likelihood of response to treatment. For dogs treated with MTDC median progression-free interval (PFI) was 17 days (range, 0–481), the median overall survival time (OST) 28 days (range, 3–421), and the 6-month and 1-year survival rates were 9.4% and 3.1%, respectively. On multivariable analysis, monocytosis and peripheral blood involvement were significantly associated with shorter PFI and OST. Long-term survival (≥100 days) was significantly associated with intermediate lymphocyte size on cytology. Dogs with LGLL have moderate response rates to chemotherapy but poor overall survival. Additional studies are needed to further evaluate prognostic factors and guide optimum treatment recommendations.

KEYWORDS
dog, haematopoietic, neoplasia, oncology, veterinary

1 | INTRODUCTION

Canine lymphoma is a heterogeneous disease that can present with variable clinical presentation, morphology, and behaviour. Classification of lymphoma subtype using the World Health Organisation (WHO) or updated Kiel systems, based on histopathology and immunohistochemistry (IHC), can aid with treatment choice and prognostication.[1] Immunophenotype largely depends on anatomic form with B-cell lymphoma being most common in multicentric forms, and T-cell subtype most common in gastrointestinal, mediastinal and epitheliotropic cutaneous forms.[2–6] As histopathology and IHC require invasive procedures such as lymph node excision or biopsy, cytopathology is considered a legitimate and less time-consuming diagnostic technique for the diagnosis of lymphoma although

limitations include inability to assess nodal architecture or accurate mitotic index (MI).[7]

Large granular lymphocytes (LGLs) are a subtype of intermediate to large lymphocyte that contain characteristic azurophilic cytoplasmic granules; they represent either cytotoxic T-cells (T-LGL) or natural killer cells (NK-LGL).[8,9] Granular lymphocyte neoplasia in dogs most commonly arises in the form of chronic lymphocytic leukaemia (CLL). Canine CLL demonstrates a T-cell immunophenotype in 44%–73% of cases, with up to 74% exhibiting LGL morphology, and typically follows an indolent course with a median overall survival time (OST) of 930 days reported.[10,11]

Canine LGL lymphoma (LGLL) is rarely described; across 13 cited publications, 23 cases of LGLL are reported.[12–24] Of these, hepatosplenic origin was most common, affecting 14 dogs, and most likely arising from CD11d + TCRγδ-restricted LGLs of the splenic red pulp.[13–15,19] Other primary sites described include peripheral lymph nodes, skeletal muscle, mediastinum, skin, urinary bladder, small intestine, spinal cord, eye, pericardium, kidney, and spleen. As clinical presentation and treatment protocols in the present literature vary, it is not currently possible to determine an optimum treatment approach for canine LGLL. Nevertheless, canine LGLL seems an aggressive form of lymphoma with only 1 dog surviving more than 195 days with chemotherapy treatment.[12–21]

Due to the rare nature of this lymphoma subtype, the primary aim of this study was to assess the largest cohort of canine LGLL cases to date to better characterise clinical presentation, cytologic features, biologic behaviour and outcomes. A secondary aim was to assess clinicopathologic data for relevant prognostic factors.

2 | MATERIALS AND METHODS

2.1 | Case selection

Medical records from the Royal Veterinary College's Queen Mother Hospital for Animals (United Kingdom) from 2005 to 2023 were reviewed for dogs with a confirmed diagnosis of LGLL based on cytology or histopathology. Large granular lymphocytes were defined as intermediate (nuclei 1.5–2.0× red blood cell [RBC] diameter) to large (nuclei >2× RBC diameter) lymphocytes containing azurophilic cytoplasmic granules as previously described.[9] Dogs were excluded if the neoplastic population comprised small lymphocytes or if there was only peripheral blood (PB) or bone marrow (BM) involvement. Ethical approval for the study was granted by the institute's ethical review board (URN SR2023-0070).

2.2 | Clinical data

Clinical information compiled included age, sex, neuter status, breed, bodyweight, presenting signs and physical examination findings. Clinicopathologic and diagnostic imaging data at diagnosis, and follow-up visits where available, were collected.

Full staging was performed at the attending clinician's discretion but was not required for inclusion. Due to the limitations of retrospective data analysis, incomplete staging, and frequent multifocal organ or tissue involvement, a clinical stage according to the World Health Organisation's (WHO) staging system was not assigned to each case.[25] Lymphoma primary anatomic site was retrospectively assigned to each case where there was sufficient information, based on the predominant location of tumour burden from imaging and cytologic/histopathologic data. Where there was multifocal organ or tissue involvement in absence of a predominant location of tumour burden, the lymphoma was classified as 'disseminated'. When possible, the presence or absence of PB and/or BM infiltration was recorded. All cases were categorised as substage *a* or *b* according to the WHO system.

Data regarding treatment were collected including induction and rescue chemotherapy protocols, and response to treatment. For dogs receiving chemotherapy, treatment response was categorised according to the Veterinary Cooperative Oncology Group (VCOG) response evaluation criteria for peripheral nodal lymphoma in dogs (v1.0) for those with peripheral nodal LGLL, or the VCOG response evaluation criteria for solid tumours in dogs (cRECIST v1.0) for those with extranodal forms.[26,27] Responses based only on clinical improvement were defined as a 'clinical response'. There was no minimum duration of response required for a dog to be classed as a responder.

Histopathology reports, where available and written by a board-certified pathologist, were reviewed for information including morphologic features, grade, and results of IHC stains. Low-, intermediate-, and high-grade lymphoma was defined based on MIs of 0–5, 6–10, and >10 per single 40× objective as previously described.[28] Additionally, results of polymerase chain reaction for antigen receptor rearrangements (PARR) and flow cytometry (FC) were obtained.

The progression-free interval (PFI) was defined as the time between start of treatment and disease progression, and survival time (ST) as the time between diagnosis and death. At the time of data abstraction each patient was recorded as alive, dead, or lost to follow-up (LTFU). In dogs treated with chemotherapy, long-term survival was defined as a ST ≥100 days as this timepoint signified the start of the plateau in the right tail of the Kaplan–Meier survival curve and represented approximately 20% of cases.

2.3 | Cytologic review

Where available, modified Wright-stained slides from each case were reviewed by a board-certified clinical pathologist (E.J.H). The following features were documented: (a) neoplastic lymphocyte size (intermediate [nuclei 1.5–2.0× RBC diameter] vs. large [nuclei >2× RBC diameter]), (b) nuclear shape (round, round-indented, indented, indented-complex, complex), (c) nucleoli (present vs. absent), (d) cytoplasmic vacuolation (none, low, moderate, marked), (e) granule size (fine, small, medium or large based on the largest granule size documented in each sample), (f) number of granules per cell (0–5, 6–10, 11–15, or 16–20 based on the highest count in each sample),

and (g) percentage of neoplastic lymphocytes that displayed cytoplasmic granulation.

2.4 | Statistical analyses

Frequency and proportion were used to report categorical variables. The Shapiro–Wilk test was used to assess normality of continuous data, which was reported as mean and standard deviation (SD) for normally distributed data, and median and range for non-normally distributed data. Kaplan–Meier product of survival probabilities was used to assess PFI and OST for the population and different cohorts of interest. Univariable and multivariable backward stepwise Cox regression analysis was used to evaluate predictors of progression and survival. Variables significant at $p \leq .10$ in univariable analysis were included in multivariable analysis where variables were retained at $p \leq .05$. Results were presented as the hazard ratio (HR) and 95% confidence intervals (CIs). Fisher's exact, chi-squared and binary logistic regression tests were used to analyse the likelihood of response to chemotherapy treatment, and whether chemotherapy-treated dogs were long-term survivors or not. For the purposes of statistical analysis patients demonstrating complete (CR) or partial (PR) response according to cRECIST v1.0 or the VCOG response evaluation criteria for peripheral nodal lymphoma, and those demonstrating a 'clinical response', were classified as responders. Results were presented as odds ratio (OR) and 95% CI, and $p \leq .05$ was considered significant. For response, progression, and survival analysis, variables assessed included: age, WHO substage, individual haematologic and biochemical abnormalities, involvement of BM or PB, primary anatomic site, presence of neoplastic effusion, individual cytologic features as described above, inclusion of L-asparaginase in chemotherapy protocol, treatment with lomustine-based chemotherapy protocol, inclusion of procarbazine in lomustine-based chemotherapy protocol, and response to treatment. Dogs who were alive at the time of data collection, LTFU, or were euthanised at the time of diagnosis were censored from survival analysis. All statistical analyses were performed using SPSS Statistics version 29.0 (IBM, Armonk, NY, USA).

2.5 | Cell line validation statement

Cell line validation was not conducted because cell lines were not used in this retrospective study.

3 | RESULTS

3.1 | Patient characteristics

A total of 67 dogs were initially identified in the medical database. Two dogs were subsequently excluded due to having only PB involvement, one of which also demonstrated the small–intermediate lymphocyte size. Sixty-five dogs were therefore finally included. The median age was 8.9 years (range, 1.3–14.8) and mean bodyweight 23.3 kilograms (SD, 11.0). There were 10 entire males (15.4%), 23 neutered males (35.4%), 3 entire females (4.6%) and 29 neutered females (44.6%). Twenty-eight breeds were represented, the most common being Labrador retriever ($n = 19$ [29.2%]), crossbreed ($n = 5$ [7.7%]), border collie ($n = 4$ [6.2%]), golden retriever ($n = 4$ [6.2%]), and Staffordshire bull terrier ($n = 4$ [6.2%]).

The median duration of clinical signs prior to presentation was 14 days (range, 0–72). The most common presenting clinical signs were lethargy ($n = 39$ [60.0%]), hyporexia ($n = 36$ [55.4%]), vomiting ($n = 22$ [33.9%]), weight loss ($n = 18$ [27.7%]), diarrhoea ($n = 16$ [24.6%]), polyuria ($n = 10$ [15.4%]) and polydipsia ($n = 10$ [15.4%]). Sixty-one dogs (93.8%) were classified as WHO substage *b*, and 4 dogs (6.2%) were substage *a*.

3.2 | Clinicopathologic findings

Haematology was performed in 62 dogs with the most common abnormalities being anaemia ($n = 24$ [38.7%]), neutrophilia ($n = 24$ [38.7%]) and monocytosis ($n = 23$ [37.1%]). All cases had manual blood smear evaluation performed. Circulating neoplastic lymphocytes were identified in 16 dogs (25.8%). Serum biochemistry was performed in 63 dogs and the most common abnormalities were hypoalbuminaemia ($n = 40$ [63.5%]), increased alanine aminotransferase ($n = 37$ [58.7%]), hyperbilirubinaemia ($n = 36$ [57.1%]) and increased alkaline phosphatase ($n = 35$ [55.6%]). Ionised calcium was measured in 43 dogs and was increased in only 1 dog (2.3%). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were assessed in 22 dogs, with abnormalities detected in 10 dogs (45.5%). A summary of clinicopathologic abnormalities is presented in Table 1.

3.3 | Diagnosis, staging and cytologic evaluation

Diagnostic imaging was performed at diagnosis in all 65 dogs, with bicavitary imaging performed in 40 cases (61.5%). A primary anatomic site was possible to determine in 58 dogs (89.2%). These included hepatosplenic ($n = 19$ [32.8%]), gastrointestinal ($n = 12$ [20.7%]), disseminated ($n = 12$ [20.7%]), mediastinal ($n = 5$ [8.6%]), peripheral nodal ($n = 2$ [3.4%]), pulmonary ($n = 2$ [3.4%]), and 1 (1.7%) each of hepatic, renal, nasal, peripheral nervous system (bilateral trigeminal nerves), central nervous system (CNS; brain), and pericardial locations. Neoplastic effusions were noted and confirmed with cytology in 17 dogs (26.2%); 10 dogs (15.4%) had peritoneal effusion, 5 dogs (7.7%) had pleural effusion, 1 dog (1.5%) had both peritoneal and pleural effusion, and 1 dog (1.5%) had pericardial effusion. Twenty dogs (30.8%) had PB ($n = 15$) or BM involvement ($n = 4$), or both ($n = 1$).

Cytology was performed in 64 dogs and diagnostic for LGLL in 63 (Figure 1). In 2 dogs, diagnosis was based on histopathology. Nine dogs had both cytology and histopathology performed. On histopathology, azurophilic cytoplasmic granules were only identified in 1 case

TABLE 1 Common (frequency >10%) haematologic, biochemical and coagulation abnormalities at the time of diagnosis in dogs with large granular lymphocyte lymphoma.

Abnormality	Number of dogs	Percentage (%)
Haematology ($n = 62$)		
Anaemia	24	38.7
Neutrophilia	24	38.7
Monocytosis	23	37.1
Lymphopenia	21	33.9
Thrombocytopenia	17	27.4
Circulating neoplastic lymphocytes	16	25.8
Neutropenia	8	12.9
Biochemistry ($n = 63$)		
Hypoalbuminaemia	40	63.5
Increased ALT	37	58.7
Hyperbilirubinaemia	36	57.1
Increased ALP	35	55.6
Total hypocalcaemia	26	41.3
Hypoglobulinaemia	21	33.3
Hypocholesterolaemia	17	27.0
Hyperphosphataemia	13	20.6
Increased amylase	13	20.6
Increased creatine kinase	13	20.6
Hyperlactataemia	13	20.6
Increased urea	13	20.6
Hypoglycaemia	12	19.1
Increased C-reactive protein	11	17.5
Hyperchloraemia	7	11.1
Hypercholesterolaemia	7	11.1
Coagulation ($n = 22$)		
Prolonged PT and APTT	4	18.2
Prolonged PT only	3	13.6
Prolonged APTT only	3	13.6

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase.

(9.1%). Fifty-one cases had cytology available for review, and results are summarised in Table 2.

Histologic grade was possible to determine in 8 dogs; LGLL was classified as low-grade in 7 cases (87.5%) and intermediate-grade in 1 case (12.5%). IHC was performed in five cases and included CD3, CD18, CD79a and phosphotungstic acid-haematoxylin (PTAH). Polymerase chain reaction for antigen receptor rearrangements and FC were performed in 4 and 8 cases, respectively. In cases where an immunophenotype was established ($n = 16$ [24.6%]) via either IHC, PARR or FC, a T-cell immunophenotype/clonal T-cell receptor gamma chain gene rearrangement was confirmed in 13 dogs (81.3%) and a null-cell immunophenotype was confirmed in 3 dogs (18.7%). Table 3 summarises the immunophenotypic data.

3.4 | Treatment and outcomes

Twenty-two dogs were euthanised at the time of LGLL diagnosis. Forty-three dogs were treated, with 32 dogs receiving maximum tolerated dose chemotherapy (MTDC) and 11 prednisolone only. Two dogs underwent surgery prior to medical treatment; one dog with disseminated LGLL underwent palliative enucleation of an affected eye, and one dog with a jejunal mass underwent enterectomy. Median time from diagnosis to start of treatment was 2 days (range, 0–28).

Regarding those treated with MTDC ($n = 32$), 22 dogs (68.8%) received a lomustine-based protocol. These included: lomustine, vincristine, procarbazine, prednisolone (LOPP) (n = 4); LOPP with L-asparaginase ($n = 4$); lomustine, vincristine, prednisolone (LOP) ($n = 2$); LOP with L-asparaginase ($n = 3$); lomustine, prednisolone (LP) ($n = 2$); LP with L-asparaginase ($n = 2$); cytarabine, vincristine, lomustine, prednisolone ($n = 2$); L-asparaginase, cytarabine, lomustine, prednisolone ($n = 1$); cytarabine, lomustine, prednisolone ($n = 1$); L-asparaginase, lomustine, doxorubicin, prednisolone ($n = 1$). Non-lomustine-based protocols comprised: vincristine, cyclophosphamide, doxorubicin, prednisolone (CHOP) ($n = 3$); cytarabine, vincristine ($n = 2$); L-asparaginase, vincristine, cyclophosphamide, prednisolone ($n = 1$); L-asparaginase, vincristine, cytarabine ($n = 1$); L-asparaginase, vincristine ($n = 1$); vincristine ($n = 1$); cytarabine ($n = 1$).

Treatment response could be assessed in 27 dogs. A response to induction chemotherapy was documented in 20 dogs (74.1%) consisting of CR ($n = 1$ [3.7%]), PR ($n = 5$ [18.5%]), and 'clinical response' ($n = 14$ [51.9%]). Stable disease (SD) was documented in 1 dog (3.7%) and progressive disease (PD) in 6 dogs (22.2%). No data regarding treatment response was available for dogs treated only with prednisolone.

Nine dogs received a rescue protocol at the time of disease progression. Protocols included: doxorubicin, prednisolone ($n = 2$); vincristine, cyclophosphamide, prednisolone ($n = 1$), methotrexate, actinomycin-D, cytarabine, dexamethasone ($n = 1$); L-asparaginase, cytarabine, cyclophosphamide, prednisolone ($n = 1$); L-asparaginase, lomustine, cytarabine, prednisolone ($n = 1$); L-asparaginase, doxorubicin, cyclophosphamide, prednisolone ($n = 1$); masitinib, doxorubicin, lomustine ($n = 1$); and chlorambucil, prednisolone ($n = 1$).

Response to rescue chemotherapy could be determined in 6 dogs. A response was documented in 5 dogs (83.3%) consisting of PR ($n = 2$ [33.3%]) and 'clinical response' ($n = 3$ [50.0%]). Progressive disease was confirmed in 1 dog (16.7%).

Five dogs were LTFU with a median time to follow-up of 6 days (range, 2–48). The remaining 60 dogs were confirmed to have died from lymphoma-related causes. Median PFI, based on available data in 37 dogs, was 17 days (range, 0–481). The median OST for all dogs was 7 days (range, 0–532).

3.5 | Prognostic factors

There was a numerical but not significant difference in the median PFI and OST between dogs treated with corticosteroids only ($n = 11$) or MTDC ($n = 32$) (PFI 10 days [range, 0–481] vs. 26 days [range, 0–

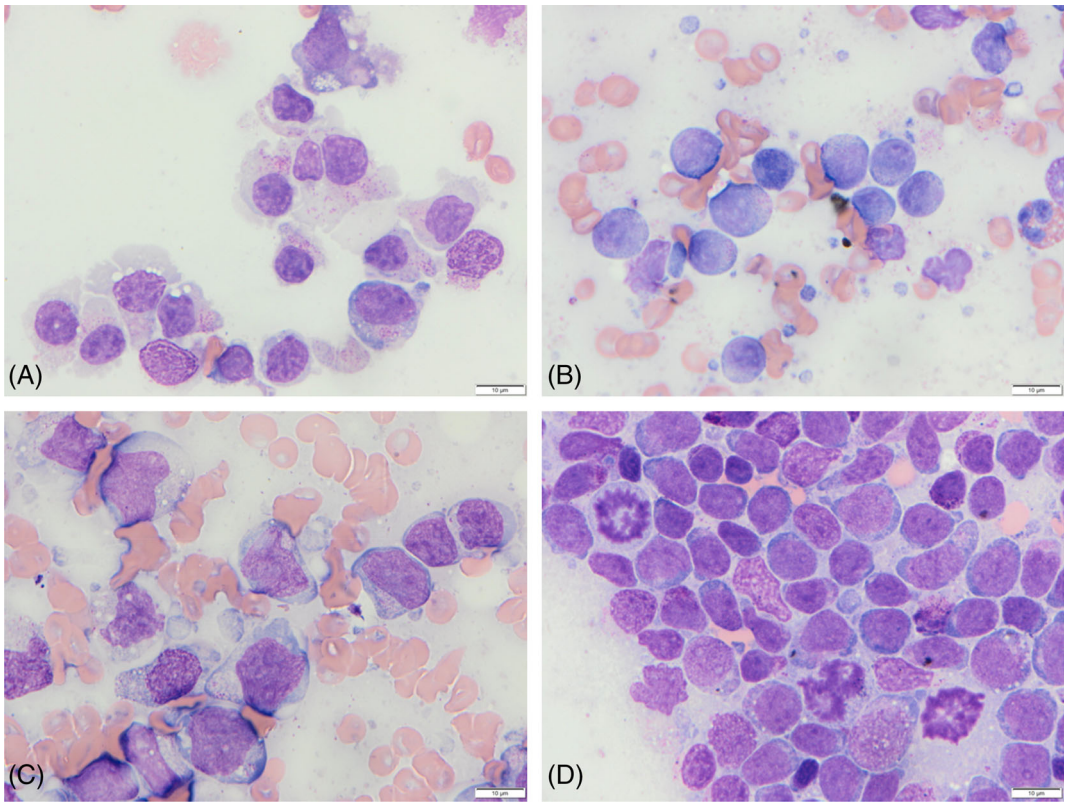

FIGURE 1 Cytomorphology of large granular lymphocyte lymphoma cases, modified Wright stain, 100× objective. (A) Intermediate lymphocytes with round nuclei, approximately 1.5 RBCs in diameter, clumped chromatin, inapparent nucleoli and a moderate amount of pale blue cytoplasm frequently containing moderate numbers of fine magenta granules. (B) Intermediate lymphocytes with round nuclei, approximately 1.5 RBCs in diameter, finely stippled chromatin, inapparent nucleoli and a small amount of dark blue cytoplasm with low numbers of cells containing moderate numbers of small magenta granules. (C) Large lymphocytes with round to indented nuclei approximately 2–2.5 RBCs in diameter, coarsely stippled chromatin, 1–2 discrete nucleoli and a moderate amount of mid-blue cytoplasm with low numbers of cells containing low numbers of small magenta granules. (D) Large lymphocytes with round to indented nuclei, approximately 2 RBCs in diameter with finely stippled chromatin, inapparent nucleoli and a small amount of dark blue cytoplasm with occasional cells containing moderate numbers of small magenta granules. Frequent mitotic figures present. RBC, red blood cell.

408], respectively [$p = .353$]; OST 7 days [range, 4–532] vs. 28 days [range, 3–421], respectively [$p = .585$]). For dogs treated with MTDC, the 6-month and 1-year survival rates were 9.4% and 3.1%, respectively.

Response to chemotherapy was significantly associated with age, neutropenia, and thrombocytopenia. Older dogs had a lower likelihood of responding to treatment ($p = .035$; OR = 0.43 [95% CI 0.20–0.94]). Specifically, dogs ≥7 years of age ($n = 19$) had reduced odds of treatment response compared to those <7 years ($n = 13$) ($p = .010$; OR = 0.56 [95% CI 0.37–0.87]). Dogs with neutropenia at diagnosis ($n = 3$) also had reduced likelihood of response compared to those without ($n = 27$) ($p = .046$; OR = 0.17 [95% CI 0.07–0.41]). Dogs with thrombocytopenia at diagnosis ($n = 7$) also had reduced likelihood of response compared to those without ($n = 23$) ($p = .028$; OR = 0.05 [95% CI 0.00–0.69]). Dogs achieving a response ($n = 20$) had a numerical but not significant improvement in the median OST compared to those who did not respond ($n = 7$) (70 days [range, 13–532] vs. 6 days [range, 5–421], respectively [$p = .099$]). No other variables were associated with achieving a response to treatment with chemotherapy.

Prognostic factors for progression and survival were assessed in dogs receiving MTDC ($n = 32$). Factors associated with PFI and OST on univariable analysis ($p \leq .10$), and therefore used in multivariable analysis, are displayed in Table 4. On multivariable analysis dogs with monocytosis ($p = .009$; HR 3.20 [95% CI 1.34–7.64]) or peripheral blood involvement ($p = .005$; HR 5.23 [95% CI 1.67–16.37]) had significantly shorter PFIs. The same variables were associated with survival; dogs with monocytosis ($p = .022$; HR 2.71 [95% CI 1.15–6.38]) or peripheral blood involvement ($p = .004$; HR 5.14 [95% CI 1.71–15.46]) had significantly shorter OSTs. No other factors were associated with PFI or OST.

Of the dogs receiving MTDC, 6 (18.8%) were classified as long-term survivors based on a ST ≥100 days. The median OST for long-term survivors was 198 days (range, 101–421). The only variable significantly associated with long-term survival was lymphocyte size. Dogs with large-cell LGLL were significantly less likely to be

TABLE 2 Cytological features of 51 cases of canine large granular lymphocyte lymphoma.

Categorical parameters		Number of dogs	Percentage (%)
Lymphocyte size	Intermediate	19	37.3
	Large	32	62.7
Nuclear shape	Round-indented	34	66.7
	Round	11	21.6
	Indented	4	7.8
	Indented-complex	2	3.9
Nucleoli	Present	16	31.4
	Absent	35	68.6
Cytoplasmic vacuolation	Absent	3	5.9
	Low	8	15.7
	Moderate	32	62.7
	Marked	8	15.7
Granule size	Fine	1	2.0
	Small	36	70.6
	Medium	13	25.4
	Large	1	2.0
Granules per cell	0–5	11	21.6
	6–10	24	47.0
	11–15	8	15.7
	16–20	8	15.7
Continuous parameters		**Median**	**Range**
Granulated neoplastic lymphocytes per case (%)		20	1–95

Abbreviation: HPF, high-powered field.

long-term survivors compared to those with intermediate-cell LGLL ($p = .021$; OR 0.05 [95% CI 0.00–0.67]).

4 | DISCUSSION

This study is the first to describe the clinical presentation, cytologic features, treatment outcomes and prognostic factors in a large population of dogs with LGLL. Similar to previous case reports or series, this study confirms the prognosis for canine LGLL is poor with a median OST of 28 days for dogs treated with MTDC.[12–24] This is similar to dogs with other aggressive forms of lymphoma such as renal lymphoma, and shorter than those with CNS or alimentary lymphoma.[29–31] There were numerical, but not statistically significant, differences in median OST between dogs treated with corticosteroids and chemotherapy, and those that responded to MTDC and those that didn't, although these may reach significance in a larger cohort.

Most (93.8%) dogs in our study were substage *b* at diagnosis, reflecting the aggressive biologic behaviour of LGLL, with extra-nodal forms and T-cell immunophenotype predominating. It is well known that, compared to multicentric B-cell lymphoma, dogs with multicentric T-cell lymphoma or extranodal forms are more likely to present clinically unwell.[32–34] Hypercalcaemia often contributes to clinical signs in dogs with T-cell lymphoma but, given the low incidence of ionised hypercalcaemia in our study (2.3%) this is less likely to contribute to the high proportion of dogs presenting with clinical signs.[7] Substage was not prognostic, possibly due to low statistical power because of the low number of cases (6.2%) presenting clinically well.

Haematological abnormalities were frequent with anaemia, neutrophilia, monocytosis and thrombocytopenia being common, which have previously been associated with poorer prognosis in canine lymphoma.[35–37] In our study, monocytosis was prognostic for shorter PFI and OST on multivariable analysis. It is unclear why monocytosis resulted in poorer patient outcome. It is possible some monocytes detected on haematology were actually myeloid-derived suppressor cells, comprising immature monocytes and neutrophils, which suppress T-cell and NK-cell mediated antitumour immunity.[38,39] Monocytosis could also be due to increased tumour production of monocyte chemotactic protein-1 (MCP-1), known to be increased in lymphoma-bearing dogs[39]; MCP-1 can recruit tumour-associated macrophages which are associated with poorer outcomes in many human cancers due to their pro-angiogenic and immunosuppressive effects.[39,40] Thrombocytopenia and neutropenia were associated with reduced likelihood of response to chemotherapy. This has previously been demonstrated in dogs with multicentric B-cell lymphoma.[35] Although the mechanism is not known, it is possible that factors such

TABLE 3 Immunophenotypic data for 16 dogs with large granular lymphocyte lymphoma.

Case	Anatomic form	Immunophenotype	PARR	IHC	Flow cytometry
1	Hepatosplenic	T-cell	Clonal TCR gene rearrangement	–	–
2	Hepatosplenic	T-cell	Clonal TCR gene rearrangement	–	–
3	Hepatosplenic	T-cell	Clonal TCR gene rearrangement	–	–
4	Disseminated	T-cell	Clonal TCR gene rearrangement	–	–
5	Not possible to determine	T-cell	Clonal TCR gene rearrangement	–	–
6	Disseminated	T-cell	–	CD3+	–
7	Gastrointestinal	T-cell	–	CD3+, CD79a−	–
8	Gastrointestinal	T-cell	–	CD3+	–
9	Mediastinal	T-cell	–	–	CD3+, CD45+, CD4−, CD5−, CD8−, CD21−, CD79a−, CD34−, MHC-II−
10	Mediastinal	T-cell	–	–	CD3+, CD45+, CD4−, CD5−, CD8−, CD21−, CD79a−, CD34−, MHC-II−
11	Not possible to determine	T-cell	–	–	CD3+, CD4+, CD5+, CD8+, CD45+, MHC-II+, CD21−, CD79a−
12	Mediastinal	T-cell	–	–	CD3+, CD45+, CD4−, CD5−, CD8−, CD21−, CD79a−, CD34−, CD11d−
13	Disseminated	T-cell	–	–	CD3+, CD45+, MHC-II+, CD4−, CD5−, CD8−, CD21−, CD79a−, CD34−
14	Renal	Null-cell	–	CD3−, CD79a−, CD18−	CD3−, CD21−
15	Gastrointestinal	Null-cell	–	–	CD45+, CD3−, CD4−, CD5−, CD8−, CD21−, CD79a−, CD34−, CD11d−
16	Disseminated	Null-cell	–	–	CD45+, CD34+ (weak), CD3−, CD4−, CD5−, CD8−, CD21−, CD79a−, MHC-II−, CD14−, MPO−, MAC387−

Abbreviations: IHC, immunohistochemistry; MPO, myeloperoxidase; PARR, polymerase chain reaction for antigen receptor rearrangements; TCR, T-cell receptor; –, not performed.

as BM involvement, immune dysregulation and increased inflammatory biomarkers may contribute.[35]

Most dogs (32.8%) in this study had primary hepatosplenic LGLL, similar to previous literature where 60.9% of reported cases are hepatosplenic in origin.[12–24] Similar to humans, hepatosplenic lymphoma in dogs is thought to arise from cytotoxic γδ T-cells within the splenic red pulp, characterised by a CD11d + immunophenotype, although assessment for γδ or CD11d expression was not performed in any of the cases of hepatosplenic LGLL in the present study.[15] Primary gastrointestinal LGLL was also common (20.7%). This is the most common form encountered in cats with LGLL, and γδ T-cells are common in epithelial surfaces so it is possible that gastrointestinal LGLLs arise from this specific T-cell immunophenotype.[41] Twenty dogs (30.8%) had PB or BM involvement. This is similar to the previously reported LGLL cases where 34.8% of dogs had PB or BM involvement, although in both our study and previously reported cases not all patients underwent full staging so the true incidence of PB or BM involvement could be higher.[12–24] Dogs with PB involvement had shorter PFI and OST on multivariable analysis. Comparing this finding to previous literature is challenging as the prognostic significance of stage V lymphoma in dogs is unclear and may vary depending on type of lymphoma[42]; whilst some studies associate stage V disease with poorer survival, other studies find no relationship between stage and outcome.[43–46] Similar to our study, PB but not BM involvement was significantly associated with survival in feline LGLL.[41] It is therefore possible that stage V disease, particularly PB involvement, has more prognostic relevance in LGLL compared to other lymphoma types and warrants further investigation in future studies.

Lymphocyte size was significantly associated with long-term survival (≥100 days); dogs with large-cell LGLL were less likely to achieve long-term survival compared to those with intermediate-cell LGLL. Although intermediate- and large-cell lymphomas are often considered together cytologically in the context of biologically aggressive lymphoma, it is possible differences in biologic behaviour and outcome exist between lymphomas of intermediate and large cell size, and this should be investigated further in future studies of both LGLL and non-LGL lymphoma.

TABLE 4 Univariable Cox regression analysis results ($p \leq .10$) included in multivariable analysis for prognostic factors for progression and survival in dogs with large granular lymphocyte lymphoma treated with maximum tolerated dose chemotherapy.

Progression				
Parameter (number of dogs)		**PFI (days)**	***p* value**	**HR (95% CI)**
Thrombocytopenia	No ($n = 23$) Yes ($n = 7$)	47 5	.028	2.74 (1.11–6.73)
Monocytosis	No ($n = 18$) Yes ($n = 12$)	48 17	.033	2.41 (1.07–5.41)
Peripheral blood involvement	No ($n = 24$) Yes ($n = 6$)	47 3	.022	3.35 (1.29–9.45)
Response to treatment	No ($n = 7$) Yes ($n = 20$)	3 49	.090	0.43 (0.18–1.13)
Survival				
Parameter (number of dogs)		**OST (days)**	***p*- value**	**HR (95% CI)**
Thrombocytopenia	No ($n = 23$) Yes ($n = 7$)	61 7	.035	2.60 (1.07–6.31)
Monocytosis	No ($n = 18$) Yes ($n = 12$)	61 38	.071	2.10 (0.94–4.71)
Peripheral blood involvement	No ($n = 24$) Yes ($n = 6$)	61 7	.014	3.63 (1.30–10.10)
Lymphocyte size	Intermediate ($n = 7$) Large ($n = 16$)	101 38	.088	2.23 (0.89–5.62)
Response to treatment	No ($n = 7$) Yes ($n = 20$)	6 70	.099	0.46 (0.19–1.16)

Abbreviations: CI, confidence interval; HPF, high powered field; HR, hazard ratio; OST, overall survival time (median); PFI, progression-free interval (median).

Despite intermediate-large cell size and aggressive biologic behaviour, 7 of the 8 LGLL cases (87.5%) where histopathology was available were low-grade based on WHO criteria.[28] This is comparable to previously reported LGLL cases; of the 12 where histopathology was performed, 10 (83.3%) were low-grade and 2 (16.7%) were high-grade.[12–16,18,23] Despite appearing to be a predominantly histologically low-grade neoplasm, canine LGLL follows a biologically high-grade clinical course with a poor prognosis. The fact only 1 of 11 cases where histopathology was performed had visible azurophilic cytoplasmic granules highlights the importance of cytology in diagnosing and recognising the clinical implications of LGLL.

Regarding immunophenotype, T-LGLs are most commonly positive for CD3, CD8 and TCRα/β although can occasionally be CD4+/CD8-, or CD4-/CD8- with TCRγδ restriction.[8,47] Natural killer LGLs are often negative for surface CD3, CD4 and CD8, and both T-LGLs and NK-LGLs are positive for granzymes B and M.[8,9] In 16 dogs where immunophenotyping was performed, LGLL was confirmed T-cell in 81.3%. Where FC was performed, 4 of 5 cases were CD4-/CD8-, and 1 was CD4+/CD8+. Assessment for CD11d was performed in one case and was negative, and no dogs had TCRγδ evaluated. Three cases were null-cell based on lack of CD3 and other lymphocyte marker expression. These cases were possibly NK-LGL origin but, as PARR was not performed to exclude T-LGL with complete loss of T-cell antigen receptor complex expression, this cannot be confirmed. In previous veterinary literature most LGLL cases are CD4-/CD8-, CD11d+ and γδ+ consistent with splenic red pulp origin, with the majority representing hepatosplenic LGLL; however, there is variation in CD4, CD8, CD11d and other marker expression between cases.[12–24] Therefore, it is currently unclear whether all LGLL cases originate in the splenic red pulp, and immunophenotype may depend on anatomic form.

The response rate to induction chemotherapy was 74.1%. Dogs showing clinical improvement were included as responders as many dogs died or were euthanised prior to the first planned re-staging, and they likely had some objective response (PR or CR) to treatment given the systemic improvement. However, as response was not confirmed in these cases, true response rate was likely over-estimated. In addition to neutropenia or thrombocytopenia at diagnosis, age ≥7 years was associated with poorer treatment response although the reason is uncertain. Dose intensity was not possible to review in this study, but it is possible lower chemotherapy doses were elected in older dogs to avoid increased risk of adverse effects. Contrary to human literature, however, veterinary studies have not found a strong association between dose intensity and outcome in lymphoma patients.[48,49] Recent literature suggests treating T-cell lymphoma with alkylating-rich chemotherapy protocols may improve outcome compared to those historically treated with CHOP.[50] In our study there was no difference in likelihood of treatment response, PFI, or OST between dogs treated with or without lomustine-based induction chemotherapy although this should be further investigated in a larger population.

The main limitations of this study are associated with its retrospective nature. Data obtained from medical records were incomplete

in some cases, and treatment and monitoring approaches were not standardised. Not all cases were fully staged, and the majority of cases did not have complete immunophenotyping. Due to the relatively small sample sizes, the power of statistical analysis may be limited. Differentiating dogs with lymphoma infiltration into PB and/or BM from those with lymphoid leukaemia can be challenging, particularly as there is lack of consensus definition. However, to minimise the risk of inadvertently including cases of leukaemia in our study, particularly LGL CLL, cases were excluded if neoplastic lymphocytes were small in size or if only PB or BM were involved.

In conclusion, this is the largest study to date evaluating canine LGLL. This is an aggressive form of lymphoma which is associated with short median PFI and OST. In our study, older dogs and those with neutropenia or thrombocytopenia were less likely to respond to chemotherapy. Monocytosis and peripheral blood involvement were associated with shorter PFI and OST. Lymphocyte size was also identified as a factor associated with long-term survival. Future research should focus on further characterising the immunophenotype of canine LGLL, and corroborating the prognostic factors identified in this study. The optimum management approach for canine LGLL is currently unknown.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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