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

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Email: katarzyna_purzycka@icloud.com**Abstract**

Canine lymphoma, as the most common haematopoietic malignancy, encompasses a group of heterogeneous diseases and even within the T-cell immunophenotype, differences in clinical presentation and responses to treatment exist. The aim of this retrospective study was to determine outcomes and prognostic factors of 107 dogs with multicentric non-indolent T-cell lymphoma (TCL) receiving lomustine-based (70%) and non-lomustine-based (30%) treatment. The majority were Labradors, Boxers, mixed-breed dogs and Dogue de Bordeaux. Eighty-six percent were substage b, 77% had mediastinal involvement, 15% had suspected bone marrow involvement and 12% had other extra-nodal sites of disease. The overall response rate to induction therapy was 80%; dogs receiving procarbazine in the induction protocol ($P = .042$), dogs with neutrophil concentration below $8.7 \times 10^9/L$ ($P = .006$) and mitotic rate below 10 per 5 high power field ($P = .013$), had greater response rates. Median progression-free survival (PFS) for the first remission was 105 days; lack of expression of CD3 on flow cytometry ($P < .0001$) and pretreatment with steroid ($P = .012$) were significantly associated with shorter PFS. Median overall survival time (OST) was 136 days; co-expression of CD79a ($P = .002$), lack of CD3 expression on flow cytometry, presence of anaemia ($P = .007$), and monocytopenia ($P = .002$) were predictive of shorter OST. Multicentric non-indolent TCL in dogs is an aggressive cancer with new possible prognostic factors.

KEYWORDS

canine, chemotherapy, lymphoma

1 | INTRODUCTION

Lymphoma is the most common canine haematopoietic malignancy.¹ Several negative prognostic factors have been identified, of which T-cell immunophenotype is one of the most consistently reported.²⁻⁹ T-cell immunophenotype represents between 13% and 38% of cases in the canine literature.^{3,8-13} Canine lymphoma classification can be

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based on clinical, morphological and immunological features. The World Health Organization (WHO) classification of human Non-Hodgkin Lymphoma has been extrapolated to canine lymphoma.¹³

Canine T-cell lymphoma (TCL) is a heterogeneous disease. It represents a wide spectrum of disease entities with varying responses to treatment and prognoses. The two extremes are illustrated by the typically indolent T-zone lymphoma (TZL)^{14,15} and the aggressive hepato-splenic lymphoma of gamma-delta T-cell lymphocytes.¹⁶ One study found that the most common types of non-indolent TCLs were peripheral TCL not otherwise specified (PTCLNOS—16%) and T-cell lymphoblastic lymphoma (TLBL—5%).¹³ Other forms of non-indolent TCL are extra-nodal, such as gastrointestinal lymphoma with modest response to chemotherapy¹⁷ or cutaneous lymphoma with variable clinical course.¹⁸ Currently, most studies on prognostic factors for canine lymphoma examine a heterogeneous population of dogs with different subtypes of lymphoma. Recently, it has been identified that more defined subpopulations of lymphoma are characterized by different prognostic factors, as in the case of multicentric diffuse large B-cell lymphoma.^{19,20} Currently, limited data on prognostic factors specific for TCL as a separate clinical entity is available. Some of the existing studies on canine TCL have included cases of indolent TZL or anatomical presentations other than multicentric, which may be a significant confounding factor for disease-free intervals and survival times as well as for prognostic factors assessments. Brodsky et al,²¹ Rebhun et al²² and Brown et al²³ did not find any statistically significant prognostic factors in cohorts of dogs with different anatomical forms of TCL treated with L-asparaginase/MOPP, CHOP and LOPP chemotherapy protocol, respectively. Another study²⁴ describing 70 dogs with non-indolent multicentric TCL treated with alkylating-agent-rich combination protocol (VELCAP-SC) found that achieving complete remission (CR) was statistically significant as an independent predictor for progression-free survival (PFS) and overall survival time (OST) and that substage b and Boxer breed were negative prognostic factors. Morgan et al²⁵ described the outcome in 35 dogs with non-indolent TCL (including hepato-splenic and gastrointestinal forms) treated with LOPP protocol. Multivariable analysis showed that Boxer breed negatively impacted PFS and dogs with multicentric lymphoma were more likely to achieve CR than other anatomical forms.

Multiple studies evaluating prognostic significance of haematologic abnormalities in human and veterinary patients with lymphoma have been published. Absolute lymphocyte concentration,^{26,27} monocyte concentration (AMC),²⁸ neutrophil:lymphocyte ratio (NLR),^{19,27,29,30} and lymphocyte:monocyte ratio (LMR)^{19,31} have been the subjects of investigations. To the authors' knowledge, these have not yet been evaluated in dogs with non-indolent TCL.

The purpose of this study was to describe patient demographics, clinico-pathological abnormalities, and outcome of dogs with multicentric non-indolent TCL. We hypothesized that within the group of non-indolent multicentric TCL treated with chemotherapy, treatment type, haematologic parameters and flow cytometry characteristics would be prognostic and that patient outcomes would be inferior to previously reported studies.

2 | MATERIALS AND METHODS

2.1 | Study design and case collection

Record of dogs with non-indolent multicentric TCL that were referred to three referral centres in the United Kingdom between January 2009 and June 2018 were retrospectively reviewed. Patients with non-indolent lymphoma were included in the study if they had cytological or histologic diagnosis of lymphoma and T-cell immunophenotype. Dogs were excluded if a definitive diagnosis was not achieved, if immunophenotyping was not available, if cytological or histopathological morphological characteristics were suggestive of a "low grade" or TZL, or if neoplastic cells were lacking expression of CD45. Dogs with non-multicentric forms such as gastrointestinal, cutaneous and primary hepato-splenic lymphomas were excluded. Clinical records were reviewed and follow-up data were obtained from existing medical records and requested from referring veterinarians by phone calls.

2.2 | Data collection

For each patient, the following data were recorded: signalment, body weight, age at diagnosis, haematology results, calcium measurements, clinical signs at presentation, method of diagnosis (cytology or histopathology) and immunophenotyping.

Haematological abnormalities were defined based on the reference intervals provided by the laboratories concerned. Blood smear reports (if available) were reviewed to assess for the presence of circulating neoplastic cells, and to differentiate between true thrombocytopenia and post-sampling platelet aggregation if automated low platelet count was reported. The abnormalities were recorded prior to any treatment initiation. Median absolute leukocyte concentrations were used to calculate the NLR and LMR ratios. Patients were described as "hypercalcemic" when free calcium levels or when total calcium levels were elevated without concurrent hyperalbuminemia and clinical signs typically associated with hypercalcemia of malignancy, such as polyuria/polydipsia were present.

When available, blood smears and cytological slides of tissue and fluid aspirates were reviewed by a single board-certified clinical pathologist (L. M. P.). For the remaining cases, information was obtained from the original cytopathology reports. Details recorded included: nuclear size, nuclear shape, nucleoli (number, size, prominence), cytoplasmic features, mitotic counts, presence of necrosis or capillaries and prominence of tingible body macrophages. Mitotic rate was defined as number of mitotic figures per five $\times 40$ or $\times 50$ high power fields (hpf). Where sufficient information was available, lymphoma was tentatively classified based on the WHO classification scheme, adapted to canine lymphoma,¹³ as well as previous publications on mediastinal TCLs in dogs.³² Cases were classified as lymphoma of granular lymphocytes (LGL) if they contained magenta

cytoplasmic granules, typically located in one focal perinuclear area. Lymphoma consisting of intermediate size nuclei (1.5-2 RBC in diameter) with finely granular chromatin and inconspicuous nucleoli were tentatively classified as lymphoblastic lymphomas (LBL). The remainder of the cases, having a large nucleus (>2 RBC) and/or prominent nucleoli, were grouped together as TCLs not otherwise specified (TCL-NOS).

The methods of immunophenotyping were recorded for each case. For cases with flow cytometry available, receptor expression patterns were recorded. Aberrant expression pattern on flow cytometry was based by previously published criteria.³³

Peripheral lymph node involvement was defined as enlargement or firm consistency of peripheral lymph nodes on physical examination or cytological/histological confirmation of lymphoma. Dogs presenting with clinical signs associated with systemic illness were classified as substage b. Full staging was performed at the attending clinician's discretion but was not required for inclusion.

Diagnostic imaging modalities and findings were recorded for each case. If internal lymph nodes were enlarged, they were classified as being involved. For liver and spleen, it was recorded whether the involvement was confirmed with cytology or histology.

Where the information was available, presence of neoplastic lymphocytes in bone marrow aspiration or in peripheral blood smears was used to classify cases as stage V according to WHO classification. All dogs included in the study were classified according to WHO staging criteria.

Treatment protocols, clinical response, date of progression, rescue treatments, date and cause of death and necropsy findings (if available) were recorded for each patient. Similar to previous studies, dogs were classified as receiving steroids if there was any history of continuous steroid administration for longer than 10 days prior to commencing chemotherapy.¹⁹

Chemotherapy induction protocols were classified as 1-LOP (lomustine- and vincristine-based, with/without L-asparaginase, procarbazine or cytarabine), 2-COP (vincristine- and cyclophosphamide-based, with/without L-asparaginase or cytarabine), 3-CHOP or CEOP where doxorubicin was replaced by epirubicin, with/without L-asparaginase or cytarabine, 4-lomustine-based without vincristine (lomustine with/without L-asparaginase or procarbazine) 5-prednisolone with/without L-asparaginase only. Subsequently, chemotherapy induction protocols were divided into lomustine-containing protocols vs others.

Response to first-line and rescue chemotherapy treatments was based on the Veterinary Cooperative Oncology Group response evaluation criteria for peripheral nodal lymphoma³⁴ and diagnostic imaging findings for cases with internal involvement only. Cases were classed as being in CR when lymph nodes (both peripheral or internal) had returned to normal size; partial remission (PR) when lymph nodes remained enlarged but had reduced in size by at least 30% and no new lesions were recognized; progressive disease (PD) was used for occurrence of new lesions or increase in size of enlarged lymph nodes by at least 20%; and stable disease (SD) as a change in size of lymph nodes which was not sufficient to be classified as PD or PR with no occurrence of new lesions. In cases of solely internal involvement

where no imaging was available to directly measure the response, a significant improvement in clinical signs was classified as PR. Response had to be sustained for a minimum of 28 days to be classified as a CR or PR. Dogs that died or were euthanized within 1 week of starting a treatment were considered as non-responders. The objective response rate was defined as the sum of the cases with a CR or a PR.

Rescue protocols were categorized as lomustine-based, doxorubicin-based, COP (cyclophosphamide, vincristine, prednisolone)-type, DMAC (dexamethasone, melphalan, Actinomycin D, cytarabine) or miscellaneous. Number of rescue protocols and responses was recorded for each patient.

PFS was defined as the period of time between treatment onset and disease progression or death from any other cause. Dogs were censored for PFS if lost to follow-up before progression occurred, if still alive and in CR at the end of the follow-up period or if progression did not occur before they died from cause other than lymphoma. OST was defined as time between treatment onset and death from any cause. Dogs were censored for OST if lost to follow-up, or still alive at the end of the study period.

2.3 | Statistical analysis

Frequency and proportion were used to summarize categorical variables; median (minimum and maximum) was used for numerical data. Fisher's exact test was used to compare sex/neutering status, stage and substage of the dogs between institutions. Kruskal-Wallis tests were used to compare body weight and age distributions between institutions. Kaplan-Meier curves were used to depict survival curves and estimated median survival time reported. Analytes with skewed results were divided into thirds (using tertile cut-off points) prior to further analysis. Univariable and multivariable Cox regression were employed to evaluate predictors of PFS and OST. Results were presented as hazard ratio (HR) and 95% confidence intervals (CI). Two sets of univariable and multivariable binary logistic regression were used to assess predictors of responders (CR + PR) vs non-responders (SD + PD), and CR vs PR of disease. Results were presented as odds ratio (OR) and 95% CI. Results of univariable analysis are placed in a supplementary material (Table S1). Variables that had *P* values <.1 in the univariable analysis were included in the multivariable analysis. Backward elimination method was used to obtain final multivariable models. Because of missing data, several models were developed with or without mitotic rate, CD3 or CD79a expression in the analysis. Significance level was set as 5%. Analyses were carried out in R 3.5.1-R Core Team (2018).

2.4 | "Cell line validation statement"

Since no cell lines were used in the current study, validation testing has not been conducted.

3 | RESULTS

3.1 | Patient population

One hundred and seven dogs met the inclusion criteria. Forty-four breeds were represented, the most common being Labrador retriever ($n = 19$, 18%), Boxer ($n = 14$, 13%), crossbreed ($n = 14$, 13%), Dogue de Bordeaux ($n = 9$, 8%), Cocker Spaniel ($n = 8$, 7%) and English Springer Spaniel ($n = 5$, 5%).

The median body weight was 26.3 kg (range 4.6–87 kg) and the median age was 6.5 years (range 1–14.8 years). There were 23 entire males, 46 neutered males, 9 entire females and 29 spayed females. There was no difference between the three institutions for sex, neutering status (P value = .82), bodyweight ($P = .11$) and age ($P = .09$) distribution of dogs.

Ninety-two dogs ($n = 92$, 86%) were classified as substage b. The most common clinical signs at presentation were anorexia/hyporexia ($n = 63$, 59%), lethargy ($n = 60$, 56%), polyuria/polydipsia ($n = 39$, 36%), vomiting and/or diarrhoea ($n = 36$, 34%) and respiratory signs ($n = 20$, 19%).

3.2 | Diagnosis and cytology review

Eighty-five dogs (79%) were diagnosed based on cytological review of tissue aspirates, 7 (7%) on histopathologic examination and 15 (14%) had both. T-cell immunophenotype was determined by flow cytometry in 57 dogs (53%), by demonstration of a clonal population of T cells by polymerase chain reaction for antigen receptor rearrangement (PARR) in 31 (29%), immunohistochemistry in 17 (16%) and immunocytochemistry in 2 (2%). Among 57 dogs who had flow cytometry, 31 (54%) dogs had a non-aberrant immunophenotype and 26 (46%) dogs had at least one aberrant expression pattern; among which 7 dogs had co-expression of CD4 and CD8, 9 dogs had loss of both CD4 and CD8, 6 dogs had loss of CD3 expression and 7 dogs had additional co-expression of B-cell markers (CD21 or CD79a).

Sixteen dogs (15%) were anaemic (Hct < 37%) and 29 (27%) were thrombocytopenic (PLT < $150 \times 10^9/L$) at initial diagnosis. Ten dogs (9%) were neutropenic (neutrophil concentration < $3 \times 10^9/L$), 41 (38%) were lymphopenic (lymphocyte concentration < $1.3 \times 10^9/L$) and 3 (3%) were monocytopenic (monocyte concentration < $0.15 \times 10^9/L$) at presentation. Calcium measurement was available for 106 cases. Fifty-seven dogs (53%) were shown to be hypercalcemic prior to initiation of treatment.

From 100 dogs diagnosed on cytology, samples of 46 were available for review. For another 47 cases, detailed information could be retrieved from the original reports. For remaining seven cases, only limited reports with final diagnosis were available. Based on the cytological assessment, 73 cases were classified as TCL-NOS, 10 as LBL, 4 as LGL and 13 cases did not have enough information to be further subclassified. In the seven cases that were diagnosed via histopathology only, information on diagnosis was obtained from the original reports.

TABLE 1 Results of staging

Peripheral lymph node involvement	92 (86%)
Involvement of mediastinal and abdominal lymph nodes only	15 (14%)
Diagnostic imaging of thorax and abdomen	83 (78%)
Thoracic Imaging using	
Radiographs	43 (40%)
Computed tomography	35 (33%)
Ultrasound	5 (5%)
Documented mediastinal involvement	64/83 (77%)
Abdominal Imaging using	
Ultrasound	44 (41%)
Computed tomography	36 (34%)
Radiographs	3 (3%)
Enlargement of abdominal lymph nodes	36/83 (43%)
Fine needle aspiration of the spleen performed	38/83 (46%)
Splenic involvement confirmed on cytology	20/83 (24%)
Fine needle aspiration of the liver performed	38/83 (46%)
Hepatic involvement confirmed on cytology	22/83 (27%)
Bone marrow involvement	
Suspected	16 (15%)
Confirmed on bone marrow aspirate	7 (7%)
Extra-nodal involvement	13 (12%)
Kidney	5 suspected, 1 confirmed
Lung	4 suspected
Lytic lesion of the skull	1 suspected
Lytic lesion of the scapula	1 confirmed
Subcutaneous mass	1 confirmed
Urinary bladder	1 confirmed

3.3 | Staging

Results of staging are summarized in Table 1. According to WHO staging system, 58 dogs (54%) were categorized as at least stage III, 18 (17%) as stage IV and 31 (29%) as stage V.

3.4 | Treatment and response rates

Two (2%) dogs were euthanized without any treatments and these dogs were excluded from the PFS and OST analysis. Seven dogs (7%) received prednisolone prior to commencing chemotherapy treatment. One hundred and five dogs received chemotherapy induction protocols as follows: 75 dogs (70%) received a lomustine-based chemotherapy protocol including 73 (68%) LOP and 2 (2%) lomustine-based protocols without vincristine. Eighteen dogs (17%) received COP

protocol, 7 dogs (7%) received CHOP or CEOP protocol and 5 dogs (5%) received prednisolone with addition of L-asparaginase.

Among 105 dogs undergoing chemotherapy treatment, 59 dogs (56%) achieved CR, 25 (24%) achieved PR, 7 (7%) had SD and 14 (13%) experienced PD. The overall response rate (ORR, %CR and %PR) was 80%. For dogs receiving a lomustine-based chemotherapy protocol, 46 dogs (61%) achieved CR, 19 (25%) achieved PR with the ORR of 86%.

Univariable analysis of factors significantly associated with lack of response to treatment and negative factors associated with PR vs CR are presented in the supplementary file (Table S1).

Only nuclear shape ($P = .017$) and L/M ratio above 2.3 ($P = .01$) remained statistically significant in the multivariable analysis (Table 2).

3.5 | First PFS and subsequent treatments

After induction treatment, median PFS was 105 days (range 1-1677). Univariable analysis of the factors significantly associated with shorter

TABLE 2 Multivariable analysis of induction response

	OR (95% CI)	P value
Model 1 responders vs non-responders (n = 58)		
Lack of procarbazine in the induction protocol	8.5 (1.3, 91.6)	.0419
Neutrophil concentration > 8.7	12.1 (2.3, 89.1)	.0062
Mitotic rate 5-10 vs < 5	2.0 (0.2, 22.7)	.5305
Mitotic rate > 10 vs < 5	15.1 (2.1, 173.4)	.0130
Model 2 responders vs non-responders (excluding mitotic rate; n = 104)		
Neutered vs entire	0.2 (0.1, 0.8)	.0195
Lack of procarbazine in the induction protocol	11.2 (2.8, 64.2)	.0021
Neutrophil concentration > 8.7 vs < 5.4	9.8 (2.5, 47.0)	.0020
Model 3 complete remission vs partial remission (n = 70)		
Nuclear complexity: cleaved complex vs round mildly indented	5.3 (1.4, 23.4)	.0171
L/M ratio > 2.3 vs < 1	8.9 (1.9, 57.1)	.0099

TABLE 3 Multivariable analysis of progression free survival

	HR (95% CI)	P value
Model 1 including CD3 (n = 56)		
Lack of CD3 expression	8.7 (3.1, 24.8)	<.0001
Steroids prior to diagnosis/treatment	5.3 (1.4, 19.4)	.0123
Model 2 excluding CD3 (n = 104)		
Presence of monocytopenia	9.6 (2.7, 33.8)	.0004
Steroids prior to diagnosis/treatment	3.3 (1.4, 8.2)	.0088
Neutrophil concentration > 8.7 vs < 5.4	2.2 (1.2, 3.9)	.0087

TABLE 4 Multivariable analysis of overall survival

	HR (95% CI)	P value
Model 1 excluding both CD3 and CD79a (n = 89)		
Nuclear complexity: cleaved complex vs round mildly indented	1.7 (1.1, 2.7)	.0244
Presence of anaemia	2.8 (1.5, 5.2)	.0009
Neutrophil concentration > 8.7 vs < 5.4	2.9 (1.5, 6.1)	.0027
Model 2 including CD79a (n = 48)		
Presence of CD79a expression	5.4 (1.9, 15.1)	.0015
Presence of anaemia	3.7 (1.4, 9.6)	.0067
Presence of monocytopenia	23.0 (2.9, 177.5)	.0026
Model 3 including CD3 (n = 56)		
Lack of CD3 expression	4.2 (1.6, 10.9)	.0033
Presence of anaemia	2.5 (1.1, 5.7)	.0291

PFS is included in the supplementary file (Table S1). Since not all of the lymphomas were investigated with flow cytometry, expression of CD3 was recorded in 56/107 dogs (52%) only. Risk factors associated with shorter PFS in the multivariable models of either including or excluding CD3 expression are presented in Table 3.

Following relapse, 47 dogs (44%) received a rescue chemotherapy treatment. Rescue chemotherapy included lomustine-based protocols in 13 dogs (28%), doxorubicin-based protocols in 13 dogs (28%), COP type protocol in 9 dogs (19%), DMAC in 5 dogs (10%) and other protocols in 7 dogs (15%).

Response to the first rescue treatment was recorded for 43/47 dogs. Overall response rate (CR and PR) to the first rescue treatment was 57%. There were 10 CR (21%), 17 PR (36%), 7 SD (15%) and 9 PD (19%). Median PFS after the first rescue treatment was 53 days (range 19-147). Twenty dogs (19%) received a second rescue chemotherapy protocol.

3.6 | Overall survival

Overall median survival time (MST) was 136 days (range 1-1677). Six dogs were alive at the end of the study period, and one was lost to follow-up. Seventy-five dogs had died or been euthanized because of lymphoma and 23 dogs had been euthanized for another reason including owner's preference with no clear indication of lymphoma progression (7 dogs), chemotherapy-related complications (5 dogs), pancreatitis (3 dogs), renal failure (2 dogs), sudden onset of lameness (1 dog) or paraplegia (1 dog), half body radiation therapy-related complications (1 dog), mammary carcinoma (1 dog), hemangiosarcoma (1 dog) and ruptured splenic mass (1 dog).

Factors associated with shorter OST on univariable analysis are presented in the supplementary file (Table S1). Multivariable analyses of excluding either CD3 or CD79a or both factors were presented in Table 4.

4 | DISCUSSION

In this study, we describe the clinical and pathological findings from a homogenous population of dogs with non-indolent multicentric TCL. We found that the median PFS and OS of the whole group were lower than previously reported for dogs with TCL,²¹⁻²⁵ which suggests that prognosis for this lymphoma subtype is worse than described for TCL in general. However, a selection bias in the present study owing to case recruitment solely from specialist referral centres cannot be ruled out, and could explain why our study population contained a large proportion of patients in clinical stage IV and V, with a majority in sub-stage b (86%).

Stage and substage have been previously reported as prognostic in canine lymphoma^{35,36,57} but we were unable to confirm this in our study. Results from previous studies may have been confounded by a variability of heterogeneous lymphoma subtypes included but it is also possible that the number of dogs with certain factors was too low in our study to show a statistical significance.

Not surprisingly, one of the breeds most commonly represented in our study was the Boxer. Several studies showed increased prevalence of TCL in this breed.^{21,22,37,38} In a recent study by Morgan et al,²⁵ the PFS was shorter in the Boxer breed, this association was not seen in our study, possibly because of low numbers of Boxers included. More interestingly, Dogue de Bordeaux's predisposition to TCL has been previously reported among canine population in Poland,³⁸ and more recently in Australian population,³⁹ however, these studies did not exclude indolent forms of lymphoma. Dogue de Bordeaux was the fourth most commonly represented breed in our study, which might further support a possibility of this breed being truly predisposed.

A large proportion (77%) of dogs in our population had evidence of mediastinal involvement on imaging, which is the highest reported to date. Since not all dogs (83/107) underwent thoracic imaging, this number may be even higher. Starrak et al⁴⁰ reported that 64.4% of 270 dogs with lymphoma had evidence of mediastinal lymphadenopathy on thoracic radiographs. Its presence was negatively associated with PFS and OST but this likely represents the association with T-cell immunophenotype as an independent prognostic factor. Study evaluating prognostic factors in lymphoma with associated hypercalcemia⁴¹ showed that presence of a mediastinal mass negatively impacted PFS. Mediastinal involvement did not influence any of the outcomes in our study, similarly to others.²²

The response to induction chemotherapy treatment was high (80% for all chemotherapy protocols and 86% for lomustine-based protocols). This rate was similar in some studies^{21-23,25} and lower in others.²⁴ Multivariable analysis showed that inclusion of procarbazine in the induction protocol was associated with higher likelihood of response to chemotherapy. Increased expression of one of the drug transporters of the ATP-Binding Cassette superfamily, BCRP (ABCG2), was shown in canine TCL.⁴² As a main mechanism of lymphoma resistance to anthracyclines, this might explain low response rates to doxorubicin in dogs with TCL.⁴³ Lomustine and procarbazine, as alkylating agents, are not typical substrates for the ABC

transporters, which might explain their potential benefit. Although there was a benefit in PFS and OST for dogs receiving lomustine-based induction chemotherapy in our study, this difference was not statistically significant comparing to other types of treatment.

In the multivariable analysis, lack of expression of CD3 was associated with a shorter PFS. Deravi et al⁴⁴ described absence or low CD3 expression in 38/101 (38%) of dogs with multicentric TCL. However, in their study it had no statistical significance for PFS or OST. The co-expression of CD79a was associated with shorter OST in our study. Although co-expression of B-cell markers in canine TCL has been previously reported,^{14,45} its prognostic significance in other studies remains uncertain. Interestingly, 5/6 dogs that lacked CD3 expression on flow cytometry were classified as TCL-NOS on cytology; further work is needed to investigate a possible association of different aberrant immunophenotypes with lymphoma subtypes. Morphological subtype alone can influence the outcome in dogs with TCL, such as dogs with lymphoblastic subtype identified by Valli¹³ as having shorter survival. Since not all of the cytological samples were available for review, there is a possibility that an association between particular lymphoma subtypes and the outcome was not detected in this study.

Steroid administration prior to treatment with chemotherapy was associated with shorter PFS in the multivariable analysis, similarly to previous studies.⁴⁶⁻⁴⁸ The suspected mechanism of its negative impact has been correlated with induction of multidrug resistance.

On multivariable analysis, the presence of anaemia remained statistically significant for shorter survival. Although this is in accordance with previous studies,^{19,49,50} the mechanism explaining its prognostic value remains unclear. Anaemia of chronic/inflammatory disease or bone marrow infiltration was most commonly suspected.

In the multivariable analysis, different factors were significantly associated with risk of not responding to the induction treatment or with shorter survival, such as neutrophil concentration above $8.7 \times 10^9/L$ and LMR above 2.3, mitotic rate above 10 per 5 hpf and nuclear shape. Absolute leukocyte concentrations and their ratios should be interpreted with caution in canine patients since they can be influenced by different aspects of systemic inflammation but can also be induced by stress. This is reflected in conflicting results found in two separate studies on canine lymphoma.^{19,27} Mitotic index has been shown as an independent prognostic factor in various malignant tumours in dogs.^{51,52} However, it should be noted that in our study, mitotic index was evaluated on cytology not histopathology samples and this variable was not available for review in all of the dogs included. Nuclear complexity is typically associated with the T-cell immunophenotype. Its prognostic value within this subgroup is more difficult to explain. Studies including cytological review, ideally matched with histopathological samples would be required to further investigate this variable.

Presence of monocytopenia was significantly associated with shorter PFS and OST in the multivariable analysis. Although AMC was shown to hold a prognostic value in previous studies,^{53,54} only three dogs in our study population had monocytopenia in the pre-treatment haematology, hence this should be interpreted with caution.

This study has limitations, such as its retrospective nature. Inclusion criteria aimed to identify a homogenous population of multicentric TCL in dogs, however in one third of the study population, T-cell immunophenotype was determined only by demonstration of a clonal population of T cells by PARR. PARR is not a preferred tool for immunophenotyping as cross-lineage rearrangement has been reported.⁵⁵ Flow cytometry was shown superior over PARR for immunophenotyping,⁵⁶ however in the absence of fresh samples, PARR can be an acceptable alternative, especially in conjunction with clinical signs typical of TCL, such as hypercalcemia. Although this study aimed to evaluate a specific subtype of canine lymphoma, because of its retrospective nature, not all dogs were uniformly staged and the level of treatment received by dogs varied. Response to treatment in dogs without peripheral lymphadenomegaly was assessed mainly based on clinical signs, rather than imaging studies, which would allow a more reliable, measurable response. Despite its relatively large population size, not all of the variables were recorded for each individual. This might undermine the significance of the discovered potential prognostic factors. On the other hand, statistical evaluation of a large number of variables creates the possibility of generating false positive associations by chance. Larger prospective multicentric studies are necessary to characterize non-indolent multicentric TCL in dogs in order to explore the most effective treatment, as it will become a stronger model for the human counterpart.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the clinical data supporting the findings of this study are available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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