Canine indolent and aggressive lymphoma: Clinical spectrum with histologic correlation

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Abstract

Sixty-three dogs with newly-diagnosed lymphoma underwent complete staging and received the same chemotherapy. Diffuse large B-cell lymphoma was the leading histotype (44.4%), followed by peripheral T-cell lymphoma (20.6%). Indolent lymphomas accounted for 30.2% of cases. Most dogs with aggressive B-cell lymphoma had stage IV disease. Dogs with indolent and aggressive T-cell lymphoma had more often stage V disease and were symptomatic. Liver and bone marrow were predominantly involved in B-cell and T-cell lymphoma, respectively. The clinical stage was significantly related to substage, sex and LDH levels. Aggressive B-cell lymphomas were more likely to achieve remission. Median survival was 55 days for aggressive and indolent T-cell lymphoma, 200 and 256 days for indolent and aggressive B-cell lymphoma, respectively. The prognosis of advanced indolent lymphoma does not appear to be appreciably different from that of aggressive disease. Familiarity with the various histotypes is critical to make the correct diagnosis and drive therapy.

Key words: lymphoma, dog, clinical stage, histological subtype

Introduction

Lymphoma is a generic term used to describe a heterogeneous disease entity, encompassing a number of subtypes of varying malignancy.[1] While diagnosing lymphoma as a general entity can be relatively straightforward, describing the different subtypes and anticipating their biologic behaviour can be challenging.

In human oncology, the classification of non-Hodgkin's lymphoma has changed and evolved over the years. Established in 1994, and updated in 2000, the Revised European American Lymphoma (REAL) classification system is the latest attempt to list well identifiable entities with a significantly distinctive biologic behaviour.[2,3,4] This model has become the basis of the World Health Organization (WHO) system in place today in dogs as well.[5]

In veterinary medicine, the morphologic diagnosis of canine lymphoma has historically relied on cytological details.[6,7] The value of histopathological analysis has been deemed necessary to define those entities requiring a structural evaluation of the nodal or splenic architecture, such as the indolent lymphomas.[8-10] A recent retrospective study investigated survival of dogs undergoing treatment for specific lymphoma histotypes, stressing the need for a precise pathological diagnosis, leading to tailored chemotherapeutic protocols.[11] Indeed, with the increasing knowledge of cancer biology, veterinary oncologists must be aware that the complexity of lymphoma classifications and the prognostic importance of architectural assessment in lymphoid neoplasms may advocate excisional biopsy to confirm and better characterize a primary cytological diagnosis of lymphoma, to accurately determine prognosis and to guide therapy.

In the last years, heavy emphasis has been placed on clinical staging of canine lymphoma,[12-14] and substantial effort has been made to classify biopsies according to

the WHO classification.[5] However, no extensive data exist in the literature with respect to the correlation between histological subtypes of canine lymphoma, clinical data and outcome.

Aims of this study were: a.) to assess whether specific histological subtypes are more consistently related to clinical stages than others; b.) to assess whether within clinical stages the histological subtypes manifest differences in anatomic distribution of disease; c.) to identify correlations between specific clinico-pathological variables and histological subtype or clinical stage; and d.) to evaluate whether treatment response and survival rates vary with histological subtypes, even within clinical stages and if, within histological subtypes, survival time is influenced by obtainment of remission.

Materials and methods

Dogs with newly-diagnosed, previously untreated lymphoma admitted to the Centro Oncologico Veterinario between June 2011 and February 2013 were prospectively enrolled.

To be eligible for recruitment, dogs were required to undergo a complete staging work-up, consisting of history and physical examination, complete blood cell count with differential, serum biochemistry profile (including total lactic dehydrogenase [LDH] levels and ionized calcium), standard cytological evaluation of smears of involved lymph nodes (carried out by one clinical pathologist, VM) routinely stained with May-Grünwald-Giemsa, thoracic radiographs and abdominal ultrasound or total-body CT (TBCT) scan according to the site of presentation, cytological evaluation of liver and spleen regardless of the ultrasonographic appearance, immunophenotype determined by

flow cytometry on a lymph node aspirate, peripheral blood and bone marrow (BM) aspirate, and histopathological and immunohistochemical evaluation of a surgically removed, enlarged lymph node (carried out by one pathologist, LA).

Immunophenotyping by flow cytometry was performed on lymph node aspirate collected in RPMI 1640, peripheral blood and BM collected in EDTA tubes. A panel of antibodies was used for cellular labelling, as previously described,[15] and included CD45 (pan-leucocyte; clone YKIX716.13, Serotec), CD3 (T-cells; clone CA17.2A12, Serotec), CD5 (T-cells; clone YKIX322.3, Serotec), CD4 (T-helper cells and neutrophils; YKIX302.9, Serotec), CD8 (T-cytotoxic cells; YCATE55.9, Serotec), CD21 (B-cells; CA2.1D6, Serotec), CD79a (B-cells; clone HM57, Serotec),), and CD34 (progenitor of hematopoietic cells; clone 1H6, Becton Dickinson). Samples were analyzed by means of a FACScalibur flow cytometer and CellQuest specific software (Becton Dickinson, San Jose, CA, USA). Hematoxylin-eosin-stained sections were examined and tumors classified according to the modified WHO criteria[5]. Immunophenotyping was determined for all cases and performed on paraffin wax-embedded sections by immunohistochemical reaction.[5] Three µm thickness serial sections mounted on Superfrost®Plus slides were used for immunohistochemistry analysis. A panel of 4 antibodies were used, including anti-CD3 antibody (clone F7.2.38, monoclonal mouse, 1:50; Dako Italia), anti-CD5 (clone CD5/54/F6, monoclonal mouse, 1:50; Dako Italia), anti-CD79acy (clone HM57, monoclonal mouse, 1:50; Dako Italia) and anti-CD20 (clone RB-9013-P, epitope specific rabbit, 1:800; Thermo Fisher Scientific Inc UK). Immunohistochemical staining was performed using the Ventana Benchmark XT (Roche, Brighton, UK). After staging work-up, dogs were assigned to a clinical stage according to the WHO staging system[16] and treated by means of the same Cytoxan- Hydroxydaunorubicin [doxorubicin]- Oncovin- Prednisone

(CHOP)-based multidrug chemotherapy (Table 1), consisting of L-asparaginase (Leunase 10000 UI; Rhône- Poulenc Rorer, Rouen, France), vincristine (Vincristina 1 mg mL^{-1}; Teva, Milan, Italy), cyclophosphamide (Endoxan; 50 mg, Baxter, Milan, Italy), doxorubicin (Adriblastina 50 mg 25 mL; Pfizer, Latina, Italy), lomustine (Cecenu 40 mg; Medac, Hamburg, Germany), and prednisone (Vetsolone 5 mg, Bayer, Milan, Italy).

The following exclusion criteria were applied: prior therapy with any cytotoxic chemotherapeutic drug and/or steroids, and primary extra-nodal lymphoma (unless neoplastic cells were found in more than one district, proving their widespread dissemination in the organism).

Written, informed consent for the collection of medical information was obtained from all owners.

Statistical analysis

Because in this series some histological subtypes were poorly represented, cases were divided based on their morphology and immunophenotype into 4 morphological groups: 1) indolent T-cell lymphomas (consisting of T-zone [TZL] and T-cell small lymphocytic lymphoma [T-SLL]); 2) aggressive T-cell lymphomas (consisting of peripheral T-cell [PTCL] and lymphoblastic lymphoma [LL]); 3) indolent B-cell lymphomas (consisting of marginal zone [MZL], follicular [FL], B-cell small lymphocytic [B-SLL], and lymphoplasmacytic lymphoma [LPL]); and 4) aggressive B-cell lymphomas (consisting of diffuse large B-cell lymphoma [DLBCL]).

142 The possible relationship between morphological group and stage was investigated with
143 Fisher exact test. Logistic regression was then performed to detect specific significant
144 differences with regards to clinical stage among the various morphological groups.
145 Logistic regression was used to verify whether within clinical stages the 4
146 morphological groups manifested differences in anatomic distribution of disease. The
147 following anatomic sites were considered as far as being involved: peripheral lymph
148 nodes (at least one), thoracic lymph nodes (at least one), abdominal lymph nodes (at
149 least one), spleen, liver, blood, BM, any other site. Differences in anatomic distribution
150 were also investigated by comparing aggressive versus indolent lymphomas regardless
151 of phenotype, and B- versus T-cell lymphomas regardless of their clinical behaviour,
152 even within clinical stages.
153 Logistic regression was also performed to determine the possible independence of
154 clinical stage and histotype from the following variables: breed (purebred or crossbred),
155 sex (male or female), age (< or ≥ 10 years), weight (< or ≥ 10 kg), substage (a or b),
156 anaemia (present or absent), thrombocytopenia (present or absent), LDH levels (≤ or >
157 300 IU/L), hypercalcemia (present or absent).
158 Kaplan-Meier curves were drawn and compared by log-rank test to assess the influence
159 of morphological groups on lymphoma-specific survival (LSS) and time to progression
160 (TTP). Initially, all cases were included in the analysis, regardless of the clinical stage;
161 thereafter cases were investigated in relation to the clinical stage. TTP was measured as
162 the interval between initiation of treatment and progressive disease (PD). Dogs not
163 experiencing PD at the end of the study and dogs lost to follow-up before PD were
164 censored for TTP analysis. LSS was measured as the interval between initiation of
165 treatment and lymphoma-related death (meaning all fatalities due to lymphoma,
166 including euthanasia). Censoring was done for dogs that were lost to follow-up, for dogs

that died from lymphoma-unrelated causes, and for dogs that were still alive at the end of the study.

Response was classified as complete remission (CR), partial remission (PR), stable disease (SD) or progressive disease (PD) based on previously published criteria.[13] Responses were required to last for at least 28 days.

Kaplan-Meier curves and log-rank test were used to evaluate the influence of the first response to therapy (CR, PR, SD or PD) on LSS within each morphological group.

Binomial logistic regression was performed to establish if the probability of obtaining CR was different among the 4 morphological groups. This analysis was carried out by including all cases first, and by analysing different stages separately thereafter.

Analyses were performed using SPSS 19.0 for Windows and differences were considered to be significant when $p \leq 0.05$.

Results

Study population

Sixty-three dogs with previously untreated lymphoma were consecutively enrolled. Signalment, clinical presentation, clinico-pathological variables, staging workup, final diagnosis and outcome for each case are listed in Table 2.

The most represented breeds were: mixed breed (n=16, 25.4%), Doberman pinscher dogs (n=5, 7.9%), Rottweiler (n=4, 6.3%), Golden retrievers (n=3, 4.8%), and Boxer (n=3, 4.8%). Several other breeds were represented in lower numbers. There were 37 (58.7%) males (of which 5 castrated) and 26 (41.3%) females (of which 20 spayed). Median age was 7 years (range, 2 to 15 years), and median weight was 28.9 kg (range, 4.3 to 69.0 kg).

At the time of initial presentation, 34 (54%) dogs were asymptomatic, and their lymphoma was suspected based on a peripheral, painless generalized lymphadenopathy. Twenty-nine (46%) dogs showed symptoms, including lethargy (n = 9), dyspnea (n = 7), loss of appetite (n = 7), difficulty in swelling (n = 4), vomiting (n = 4), polyuria/polydipsia (n = 4), weight loss (n = 2), diarrhoea (n = 2), fever (n = 1), coughing (n = 1), and melena (n = 1)

When considering clinical stage, 1 (1.6%) dog had stage I disease (substage a), 9 (14.3%) dogs had stage III disease (7 substage a, 2 substage b), 29 (46.0%) dogs had stage IV disease (22 substage a, 7 substage b), and 24 (38.1%) dogs had stage V disease (4 substage a, 20 substage b).

DLBCL was the most common histotype, accounting for 28 (44.4%) cases, followed by PTCL, accounting for 13 (20.6%) cases. Other histological subtypes included 10 (15.9%) MZL, 3 (4.8%) SLL (2 T-cell, 1 B-cell), 3 (4.8%) LL, 3 (4.8%) FL, 2 (3.2%) TZL, and 1 (1.6%) LPL. When grouping cases based on immunophenotype and histological grade (derived from morphology and mitotic rate), there were 28 (44.4%) aggressive B-cell lymphomas, 16 (25.4%) aggressive T-cell lymphomas, 15 (23.8%) indolent B-cell lymphomas, and 4 (6.3%) indolent T-cell lymphomas.
One dog with DLBCL was excluded from statistical analysis with the exception of survival analysis because it was the only case in stage I.

Thirty-eight (60.3%) dogs obtained CR, 10 (15.9%) PR, 2 (3.2%) SD, and 10 (15.9%) dogs experienced PD. Treatment response for each histotype is shown in Table 3. In 3 cases (2 aggressive B-cell lymphomas and 1 aggressive T-cell lymphoma), the first

treatment response could not be established, because their follow-up was shorter than 28 days. Forty-five (75%) dogs experienced PD during the study period, with a median TTP of 60 days (range, 0 to 423 days); 15 (25%) dogs were still in remission at data analysis closure, with a median follow up of 157 days (range, 28 to 576 days). Overall median TTP was 110 days (range, 0 to 576 days).

Thirty-seven (58.7%) dogs were dead at the end of the study due to PD, with a median LSS of 111 days (range, 5 to 530 days). Twenty-six (41.3%) dogs were censored for LSS analysis because still alive at data analysis closure or dead for lymphoma-unrelated causes, with a median follow-up of 186 days (range, 13 to 577 days). Overall median LLS was 195 days (range, 5 to 577 days).

Do some histological subtypes relate more consistently to clinical stages than do others?

Overall, all histological subtypes diagnosed in this series of dogs occurred in different clinical stages. Fisher exact test revealed a significant association between morphological group and clinical stage (p=0.000). The distributional pattern is given in Table 4. Interestingly, all dogs with indolent T-cell lymphoma and the majority (62.5%) of dogs with aggressive T-cell lymphoma had stage V disease.

Indolent lymphomas were excluded by the subsequent logistic regression analysis, because none of the dogs had stage III disease. Therefore, only differences between aggressive B-cell and T-cell lymphomas were investigated. Again, a significant association between clinical stage and morphological group was identified (p=0.009). In particular, aggressive B-cell lymphomas had a significantly higher probability of presenting in stage IV than in stage V (p=0.002) when compared to aggressive T-cell lymphomas.

Within given clinical stages, do the histological subtypes manifest differences in anatomic distributions of disease?

When considering all cases regardless of clinical stage, significant site predilections were identified. In particular, the liver was more frequently involved in aggressive B-cell and indolent T-cell lymphomas than in aggressive T-cell lymphomas (p=0.000). The spleen was more frequently involved in aggressive B-cell lymphomas than in indolent T-cell lymphomas (p=0.000), and in indolent T-cell than in aggressive T-cell lymphomas (p=0.000). BM was more frequently involved in indolent and aggressive T-cell lymphomas than in aggressive B-cell lymphomas (p=0.001 and p=0.049, respectively).

When considering stage III and IV, no differences were noted in anatomic distribution of disease. On the other hand, among dogs with stage V disease, thoracic lymph nodes were more frequently involved in aggressive B-cell lymphomas than in indolent B-cell and T-cell lymphomas (p=0.003 and p=0.027, respectively), whereas BM was more frequently involved in aggressive B-cell and T-cell than in indolent B-cell lymphomas (p=0.002 and p=0.047, respectively). Furthermore, although not statistically significant, aggressive T-cell lymphomas tended to involve extra-nodal non-lymphoid sites more frequently than the other groups.

No site predilection was detected when grouping together all aggressive and indolent lymphomas, even within clinical stages.

When grouping cases based on phenotype (T versus B), it was found that the liver was more frequently involved in B-cell lymphomas than in T-cell lymphomas (p=0.003), whereas the opposite was true when considering BM (p=0.003). No site predilection was identified between B- and T-cell lymphomas within clinical stages.

Do specific clinico-pathological variables relate to histological subtype and clinical stage?

Based on multinomial logistic regression, a statistically significant relationship was observed between morphological group and substage (p=0.000). In particular, 3 out of 4 (75.0%) dogs with indolent T-cell lymphoma and 14 out of 16 (87.5%) dogs with aggressive T-cell lymphoma showed symptoms at presentation. Conversely, only 6 out of 27 (22.2%) dogs with aggressive B-cell lymphoma and 6 out of 15 (40.0%) dogs with indolent B-cell lymphoma were symptomatic when first diagnosed.

The clinical stage was significantly related to substage (p=0.000), sex (p=0.002) and LDH levels (p=0.001). In particular, asymptomatic dogs had more frequently stage IV disease (66.7% of dogs in substage a), while symptomatic dogs had more frequently stage V disease (70.0% of dogs in substage b). Females had more commonly stage IV disease (52.0% of female dogs), whereas males rarely had stage III disease (8.1% of male dogs). Finally, dogs with high LDH levels had more frequently stage IV disease (73.9% of dogs with high LDH levels).

Do treatment response and survival rates vary with histological types, even within clinical stages? And does obtainment of remission influence survival within morphological groups?

Three cases for which response to therapy could not be defined due to the short follow-up were included only in LSS analysis.

Binomial logistic regression showed a significant difference in the probability to obtain CR among morphological groups (p=0.010). In particular, CR was significantly more likely to be achieved in aggressive B-cell lymphomas, compared to aggressive T-cell,

indolent B-cell and indolent T-cell lymphoma cases (p=0.003, p=0.018 and p=0.016, , respectively). Median TTP and LSS for each morphological group and stage are shown in Table 5.

Based on Kaplan-Meier curves and log-rank test, TTP was not different among the 4 morphological groups (p>0.05).

Log-rank test results revealed a significant difference in LSS among morphological groups (p=0.009) (Figure 1). In fact, median LSS was 55 days for aggressive and indolent T-cell lymphoma, 200 days for indolent B-cell lymphoma and 256 days for aggressive B-cell lymphoma.

Furthermore, paired comparisons revealed a significantly shorter LSS for aggressive T-cell lymphoma cases compared to aggressive and indolent B-cell lymphomas (p=0.003 and p=0.023, respectively). Within stage III, LSS was significantly different between aggressive B-cell and T-cell lymphomas (p=0.034): median LSS was 53 days (range, 23 to 459 days) for aggressive T-cell lymphomas, and was not reached for aggressive B-cell lymphomas. Within stage IV, LSS was significantly different among aggressive B-cell lymphomas (median LSS: 200 days), aggressive T-cell lymphomas (median LSS: 30 days) and indolent B-cell lymphomas (median LSS: 200 days) (p=0.002). In particular, a statistically significant difference was found between aggressive T-cell lymphomas and aggressive and indolent B-cell lymphomas (p=0.011 and p=0.002, respectively), but not between aggressive and indolent B-cell lymphomas (p>0.05). Within stage V, no significant difference in LSS was found among the various morphological groups.

Among dogs diagnosed with aggressive B-cell lymphoma, median LSS was 470 days (range, 28 to 544 days) for dogs achieving CR, 71 days for the dog achieving PR, 173 days for the dog with SD, and 20 days for the dog experiencing PD. The difference was statistically significant (p=0.000).

Among dogs diagnosed with indolent B-cell lymphoma, median LSS was not reached for dogs that achieved CR. Median LSS was 156 days (range, 109 to 235 days) for dogs that achieved PR and 45 days (range, 33 to 45 days) for dogs experiencing PD. The difference was statistically significant (p=0.001). In particular, dogs with PD had a significantly shorter LSS than dogs with CR and PR (p=0.005 and p=0.025, respectively).

Among dogs diagnosed with aggressive T-cell lymphoma, median LSS was 76 days (range, 54 to 572 days) for dogs that achieved CR, 64 days (range, 53 to 161 days) for dogs that achieved PR, 30 days for the dog with SD and 13 days (range, 5 to 55 days) for dogs with PD. The difference was statistically significant (p=0.002). In particular, LSS was longer in dogs that achieved CR compared to those with SD and PD (p=0.014 and p=0.003, respectively), and in dogs that achieved PR compared to those with PD (p=0.048).

Among dogs with indolent T-cell lymphoma, response to therapy had no influence on LSS (p>0.05).

Discussion

The generic diagnosis of ‘lymphoma’ encompasses a heterogeneous group of disease entities with respect to the morphological spectrum and clinical behaviour. It is apparent

that the classification and the proper management of canine lymphoma demand more knowledge of the disease. Since clinically distinct entities may appear cytologically similar, accurate histological classification by nodal excision is preferred.

Procedures for the routine staging of canine lymphoma at the Centro Oncologico Veterinario since 2011 have provided a unique opportunity for studying the relationship of histological subtype to anatomic distribution of lesions, clinico-pathological variables, treatment response and survival in a large series of previously untreated dogs.

Similar to other reports,[1,5,17] DLBCL was the leading histological subtype in this series, accounting for 44.4% of cases. PTCL and LL comprised 20.6% and 4.8% of cases, respectively, both higher than other reports using the WHO classification.[1,5] The proportion of indolent lymphoma reflected the 12-24.5% rate documented in previous analyses.[1,5,17]

In this study, we analysed whether the different morphological groups were related to specific differences in clinical presentation, anatomic distribution of disease, treatment response or survival. Because some histological subtypes were diagnosed only rarely, we divided histological subtypes in 4 major morphological groups based on the histological classification (indolent or aggressive) and on the immunophenotype (B or T).

A recent study supported the value of these groups.[18] By gene expression analysis, 3 molecular subgroups were identified, showing prognostic significance: high-grade T-cell lymphomas, low-grade T-cell lymphomas, and B-cell (including low-grade and high-grade) lymphomas. In our study, DLBCL and MZL were classified separately.

The questions raised in the introduction to this paper will be discussed in succession.

First, the distribution of cases according to the clinical stage allowed to derive the following information: the vast bulk of dogs with aggressive B-cell lymphoma had

366 stage IV disease; conversely, dogs with indolent T-cell lymphoma and the majority of
367 dogs with aggressive T-cell lymphoma had stage V disease, highlighting the ability to
368 progress unnoticed in the first case, and the biological aggressiveness in the second
369 case. Indolent B-cell lymphoma occurred in clinical stages IV and V without any strong
370 association.
371 What is rather surprising is the seemingly large number (38.1%) of dogs with clinical
372 stage V disease. To some degree this high proportion of dogs in stage V may be a factor
373 of "historical understaging", before the staging procedures now available and the
374 routinely performed BM analysis were used.[19]
375 This study demonstrates that there were preferred anatomical sites of involvement by
376 canine lymphoma, possibly reflecting underlying biological distinctions. The liver was
377 more frequently involved in aggressive B-cell and indolent T-cell lymphomas than in
378 aggressive T-cell lymphomas. The spleen was more frequently involved in aggressive
379 B-cell lymphomas than in indolent T-cell lymphomas, and in indolent T-cell
380 lymphomas than in aggressive T-cell lymphomas. BM was more commonly infiltrated
381 in all T-cell lymphomas than in aggressive B-cell lymphomas.
382 Within clinical stage V, dogs with aggressive B-cell lymphoma had more frequently
383 involvement of intrathoracic lymph nodes. Also, BM was more commonly infiltrated in
384 aggressive T-cell and B-cell lymphoma cases. . Finally, when stratifying cases based on
385 their phenotype, the liver was significantly more involved in dogs with B-cell
386 lymphoma, whereas dogs with T-cell lymphoma had a significant higher risk to have
387 BM involvement, regardless of the morphology (indolent versus aggressive).
388 Interestingly, aggressive T-cell lymphomas tended to involve extra-nodal non-lymphoid
389 sites more commonly than the other groups. The localisation of lymphoma presentation
390 by extra-nodal site revealed the skin to be the most common, followed by tongue, lung,

391 muscles, larynx, bone and mucous membranes. This finding is in agreement with the
392 human literature, as PTCL patients often have symptoms at presentation, BM
393 infiltration, and extra-nodal involvement.[20]
394 Among the evaluated variables, only substage was significantly related to the
395 morphological group. The majority of dogs with indolent and aggressive T-cell
396 lymphomas (75.0% and 87.5%, respectively) showed symptoms at presentation. This is
397 easily explainable in the case of high-grade T-cell lymphoma, which typically shows an
398 aggressive clinical course and harbours a poor prognosis,[18] as confirmed by the dogs in
399 this series.
400 Conversely, as previously mentioned, it is quite common for low-grade lymphomas to
401 behave in an indolent fashion and to progress slowly, showing no symptoms until they
402 are in advanced stages.[8,10] Indeed, all dogs with indolent T-cell low-grade lymphoma in
403 this series had stage V disease at presentation, thereby explaining the presence of
404 clinical signs. When considering clinical stage, it was found that symptomatic dogs had
405 more frequently stage V disease (70.0% of dogs). This is not surprising, as a more
406 advanced clinical stage indicates a widespread involvement, thereby possibly causing
407 clinical signs. Dogs with high LDH levels had more frequently stage IV disease. It is
408 documented that increased LDH activity is correlated with tumor burden in people with
409 DLBCL.[21] Our results may have been biased by the absence of early stage (I-II)
410 lymphoma cases and by the paucity of stage III cases. Whether LDH levels may prove
411 useful to differentiate between early and advanced cases remains to be determined.
412 Surprisingly, none of the other variables was correlated to morphological group or
413 clinical stage, including hypercalcemia, anemia and thrombocytopenia. Hypercalcemia
414 occurred rarely, and only in T-cell lymphomas, as already documented.[7] Peripheral
415 blood abnormalities, including anemia and thrombocytopenia, were not suggestive of

bone marrow infiltration (stage V), and this data is in agreement with a recently published document from our research group.[19]

When evaluating treatment response, it was found that aggressive B-cell lymphomas were more likely to achieve CR than all other morphological groups. There is ample precedent in the human literature for this association.[20,22,23]

Indolent lymphomas are usually not curable, as they grow too slowly to be accurately targeted by chemotherapy. Therefore, CR is rarely obtained and, even if treatment achieves a response, indolent lymphomas almost always recur.[24,25] . Notably, unlike previous studies indicating that obtainment of CR translates into prolonged survival for dogs with aggressive lymphoma.[26-28] the current work highlights that the same conclusion cannot be drawn for indolent T-cell lymphomas, whereby outcome was not anticipated by treatment response. Conversely, dogs with indolent B-cell lymphomas obtaining CR survived longer than those experiencing PD. Knowledge of these factors can be important in planning and analyzing future therapeutic trials.

In people as well as in dogs it has long been recognized that aggressive T-cell lymphomas have an inferior prognosis compared with their B-cell counterparts, attributable to a more frequent adverse clinical features at diagnosis, a lower treatment response rate, and a higher incidence of relapse.[19,21,29,30] In this study, less than 50% of dogs obtained CR, thereby confirming the low CR rate. However, attainment of CR was significantly related to a longer survival.

LSS differed among morphological groups, regardless of the clinical stage. Not surprisingly, a short survival was documented for dogs with aggressive T-cell lymphoma, whereas the longest LSS in this case series was achieved by dogs with aggressive B-cell lymphoma. It may be hypothesized that the short chemotherapy cycles administered to PTCL dogs in this study were suboptimal.[31] In fact, previous published

441 data have demonstrated that CHOP-based protocols failed to induce sustained
442 remissions for most dogs with aggressive T-cell lymphomas.[6,32] Future clinical trials
443 need to focus on subtype-specific treatment, incorporation of newer agents, or adding a
444 maintenance phase to improve the long-term outcome for PTCL dogs.
445 A striking, although not statistically significant difference was also observed between
446 indolent B-cell and T-cell lymphomas. Unlike dogs with indolent B-cell lymphoma,
447 which had a median LSS of 200 days, dogs with indolent T-cell lymphoma obtained a
448 median LSS of 55 days only, suggesting that the indolent term by histology does not
449 necessarily always translate into an indolent clinical behaviour and a long survival.
450 In the study by Valli,[8] low-grade T-cell lymphoma comprised 10 out of 66 cases and all
451 were ascribable to TZL. All dogs had stage IIIa disease. Two dogs received a CHOP-
452 based protocol, and 3 dogs prednisone as single agent. Reported overall median ST was
453 22.5 months, with none of these dogs dying due to lymphoma. More recently, Flood-
454 Knapik[10] described 75 dogs with indolent lymphoma and confirmed the survival
455 advantage of TZL cases (33.5 months). Treatment varied in this study: 25 dogs received
456 chlorambucil and prednisone, 11 received a CHOP-based protocol, 5 received
457 prednisone, and 7 dogs received no treatment. Unfortunately, no information was
458 provided regarding clinical stage at presentation for dogs with TZL, or treatment
459 response. In the most recent study by Valli and co-authors, indolent lymphomas had a
460 very long LSS.[11]
461 This apparent discrepancy in survival between our study and the previous analyses may
462 be obviously due to the relatively small number of indolent B-cell (n=15) and indolent
463 T-cell (n=4) lymphomas. However, it should be acknowledged that the different
464 outcome may also reflect differing individual characteristics and clinical stages among
465 enrolled dogs, or simply a different study population resulting from referral of dogs to

466 an oncology centre versus sending biopsies to a diagnostic laboratory.[11]

467 Overall, the clinical data suggest considerable clinical heterogeneity, with some dogs
468 showing a chronic/indolent, non-progressive clinical course, eligible for a watch-and-
469 see policy, while others have a more fulminant course and short survival, undoubtedly
470 rendering indolent lymphomas a challenging condition in terms of formulating a
471 prognosis and deciding how to manage them. Previous data have demonstrated that the
472 majority of dogs with indolent lymphomas may have a chronic presentation, with no
473 acute indication for therapy and a delay of approximately 2 years before the onset of
474 clinical signs.[11] It may be possible that dogs with indolent lymphoma are typically
475 referred to the specialist once symptoms occur and therapy is needed, and at this point
476 their outcome does not appear to be appreciably different from those with more classic
477 presentations of aggressive disease, as demonstrated by our results. This may suggest a
478 progression from indolent to aggressive disease similar to that seen in people.[24,33]
479 Indeed, the heterogeneity within the subgroup of indolent lymphomas in people has
480 been related to histological features (pattern, grading), genetic features, tumor
481 microenvironment, type of tumor spread (stage, tumor burden, BM involvement,
482 symptoms, etc.), laboratory data (LDH, anemia, microglobulin), disease modifications
483 after treatment (clinical response and minimal residual disease), and patient's status
484 (age, performance status, comorbidity).[34,35] The combination of these parameters has
485 allowed the identification of prognostic scores in human indolent lymphomas. In canine
486 lymphoma, we have just started to recognize different biologic entities, and more
487 research is required to identify prognostic factors specific for each subtype in an effort
488 to help clinicians in treatment decisions and prognostic formulation.

489 Another possible explanation for the different outcome between our and previous canine
490 studies may be attributable to an inadequate treatment strategy in the current case series.

Indeed, in people, there is a variety of treatment choices, more over depending on the severity of symptoms at presentation. A 'watchful waiting' may be proposed for patients without disease-related symptoms or adverse prognostic factors. Chlorambucil has been used as first line therapy, followed by CHOP-based protocols in case of relapse or refractory disease;[36,37] however, no consensus has been achieved on the optimal first-line or relapse treatment. In our case series, all dogs were treated by means of the same chemotherapeutic protocol, regardless of histotype and clinical stage, to avoid treatment bias in the response. We do not know whether a less dose-intense regimen would have translated into a more prolonged survival in dogs with indolent lymphoma. Nevertheless, the vast majority of dogs presented with advanced stage disease, and were symptomatic at diagnosis. Therefore, despite the "indolent" designation, the clinical picture prompted a more aggressive therapeutic intervention, as neither the clinician nor the owners felt comfortable with a "watch-and-wait" approach or metronomic chemotherapy. Newer treatment strategies, including immunotherapy, should be explored in future clinical trials.

In agreement with the human counterpart, it may be concluded that treatment approaches for one type of lymphoid malignancy are not necessarily applicable to other diseases, even of the same cell lineage. Future prospective clinical trials are warranted to establish the best treatment strategy for indolent and aggressive T-cell and B-cell lymphomas within different clinical stages to guide the clinician in the selection of the most appropriate treatment schedule.

Conclusions

In conclusion, dogs with aggressive B-cell lymphoma had more often hepatic involvement. Indolent and aggressive B-cell lymphomas showed the two longest median LSS in this series. Obtainment of CR correlated to a longer LSS in both groups. On the other hand, dogs with aggressive T-cell lymphoma were generally symptomatic at diagnosis, had often BM involvement, tended to have more often extra-nodal involvement, did not easily achieve CR, and died soon. Dogs with indolent T-cell lymphoma were symptomatic at diagnosis, had an advanced clinical stage and BM involvement. These dogs achieved CR, but the response was of short duration; LSS was also short.

As would be expected, we recognize that lymphomas are a heterogeneous group of distinct diseases, most unrelated to one another, and not a single disease with a spectrum of histologic grade and clinical behaviour. Familiarity with the various histological subtypes is also critical to make the correct diagnosis and institute the appropriate treatment.

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663

664 Table 1. Chemotherapy protocol administered to 63 dogs diagnosed with lymphoma.

Drug	**Week**								
	1	2	3	4	7	10	13	16	19
L-Asparaginase (400 UI/kg SC)	x								
Vincristine (0.75 mg/m2 IV)		x	X	x			X		
Cyclophosphamide (250 mg/m2 PO)		x					X		
Doxorubicin					x			X	

(30 mg/m2 IV)		
Lomustine		
(80 mg/m2 PO)	X	X
Prednisone		
(1 mg/kg PO until week 4, then 0.5 mg/kg PO)		

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666 Table 2. Signalment, clinical presentation, clinico-pathological variables, staging workup, final diagnosis and outcome of 63 dogs with lymphoma.

	Breed	Sex	Age (years)	Weight (kgs)	Histotype	Stage	Imaging	Involved sites	PCV	PLT	Ca	LDH	RTT	TTP	LSS
1	American Cocker spaniel	sf	10	9.1	DLBCL	Ia	tRX, aUS	pLN	wri	wri	wri	wri	CR	423	512
2	Argentinean mastiff	cm	5	58.0	DLBCL	IIIa	tRX, aUS	pLN	wri	wri	wri	wri	CR	245	392§
3	mixed	m	10	6.9	DLBCL	IIIa	tRX, aUS	pLN	wri	wri	wri	wri	CR	145*	146§
4	mixed	sf	12	14.8	DLBCL	IIIa	tRX, aUS	pLN	wri	wri	wri	wri	CR	423*	424§
5	Rottweiler	sf	5	59.7	DLBCL	IIIa	tRX, aUS	pLN	wri	wri	wri	wri	CR	300	410§
6	Rottweiler	sf	4	47.0	DLBCL	IIIa	tRX, aUS	pLN	low	low	wri	high	CR	28*	28§
7	Bull	sf	8	20.2	DLB	IVa	tRX,	pLN, spleen,	wri	wri	wri	wri	CR	35	49

	terrier				CL		aUS	liver							
8	Doberman pinscher dog	m	8	34.1	DLB CL	IVa	TBCT	pLN, tLN, aLN, spleen	wri	wri	wri	wri	CR	292*	293§
9	mixed	m	7	4.7	DLB CL	IVa	tRX, aUS	pLN, spleen	wri	wri	wri	wri	CR	457*	458§
10	mixed	m	5	11.2	DLB CL	IVa	tRX, aUS	pLN, spleen, liver	wri	wri	wri	wri	CR	241	470
11	Rottweiler	sf	8	47.9	DLB CL	IVa	tRX, aUS	pLN, aLN, spleen, liver	wri	wri	wri	wri	CR	187*	188§
12	Golden retriever	m	6	41.2	DLB CL	IVa	tRX, aUS	pLN, spleen, liver	low	wri	wri	wri	CR	44	156
13	Schnauzer	m	10	45.9	DLB CL	IVa	tRX, aUS	pLN, tLN, aLN, spleen, liver	low	wri	wri	high	CR	81	214
14	Bernese Mountain dog	sf	5	41.5	DLB CL	IVa	tRX, aUS	pLN, aLN, spleen, liver	wri	low	wri	wri	CR	291	544§
15	Great Dane	m	3	69.0	DLB CL	IVa	tRX, aUS	pLN, spleen, liver	wri	wri	wri	high	CR	137	200

16	mixed	sf	15	17.1	DLBCL	IVa	tRX, aUS	pLN, aLN, tLN, spleen, liver	wri	wri	wri	high	CR	134	141
17	mixed	m	11	23.3	DLBCL	IVa	tRX, aUS	pLN, tLN, aLN, spleen, liver	wri	wri	wri	high	CR	31	203§
18	Petit bleu	sf	11	24.6	DLBCL	IVa	tRX, aUS	pLN, spleen	wri	wri	wri	high	CR	139	147
19	Shar-pei	m	5	34.1	DLBCL	IVa	tRX, aUS	pLN, spleen, liver	wri	wri	wri	high	ND	ND	13§
20	Border collie	m	3	21.2	DLBCL	IVa	tRX, aUS	pLN, spleen, liver	wri	wri	wri	high	ND	ND	20°
21	German Shepherd	f	9	26.6	DLBCL	IVb	tRX, aUS	pLN, spleen, liver	low	wri	wri	wri	CR	53*	54§
22	mixed	sf	10	46.8	DLBCL	IVb	tRX, aUS	pLN, tLN, spleen, liver	wri	wri	high	wri	SD	124	173
23	Golden retriever	f	13	35.4	DLBCL	IVb	tRX, aUS	pLN, tLN, aLN, spleen, liver	low	low	wri	wri	PD	19	20
24	mixed	m	6	37.4	DLBCL	Va	tRX, aUS	pLN, tLN, aLN, spleen, liver, muscle	wri	wri	wri	wri	CR	137*	138§

25	Doberman pinscher dog	m	5	33.3	DLBCL	Va	tRX, aUS	pLN, PB, skin	wri	wri	wri	high	CR	118	256
26	German Shepherd	m	5	47.4	DLBCL	Vb	tRX, aUS	pLN, tLN, spleen, liver, lung	wri	low	wri	wri	CR	240	530
27	English bulldog	m	7	27.0	DLBCL	Vb	tRX, aUS	pLN, spleen, liver, BM	low	low	wri	wri	CR	192	237
28	mixed	m	6	24.1	DLBCL	Vb	tRX, aUS	pLN, tLN, spleen, liver, BM, lung	wri	wri	wri	high	PR	34	71
29	Dogue de Bordeaux	sf	5	49.2	PTCL	IIIa	tRX, aUS	pLN	wri	wri	wri	wri	CR	315	459[§]
30	Boxer	sf	6	28.5	PTCL	IIIa	tRX, aUS	pLN	wri	wri	wri	wri	PD	0	23
31	Belgian Shepherd	sf	8	22.8	PTCL	IIIb	tRX, aUS	pLN, tLN	wri	wri	wri	wri	PR	47	53
32	Cavalier King Charles	m	10	10.9	PTCL	IIIb	TBCT	pLN, tLN, aLN	wri	wri	wri	wri	CR	60	71

	spaniel														
33	Rhodesian ridgeback	sf	5	32.6	PTCL	IVb	tRX, aUS	aLN, spleen, liver	wri	low	high	high	SD	29	30
34	Doberman pinscher dog	cm	7	35.3	PTCL	IVb	tRX, aUS	pLN, tLN, aLN, spleen, liver	wri	wri	wri	high	ND	ND	13§
35	mixed	sf	14	7.9	PTCL	Vb	tRX, aUS	pLN, PB, BM, tongue	wri	wri	wri	wri	CR	572°	572°
36	Doberman pinscher dog	sf	12	29.4	PTCL	Vb	TBCT	pLN, spleen, skin, mucosae, lung	wri	wri	wri	wri	CR	70	76
37	Golden retriever	m	7	30.1	PTCL	Vb	tRX, aUS	pLN, skin	low	wri	wri	wri	CR	49	54
38	Wolfdog	m	9	37.0	PTCL	Vb	TBCT	pLN, tLN, aLN, PB, BM	low	low	high	wri	CR	159	195
39	mixed	cm	14	9.9	PTCL	Vb	TBCT	pLN, skin	low	wri	high	wri	PR	93	161
40	mixed	f	12	27.9	PTCL	Vb	tRX, aUS	pLN, spleen, PB	low	low	wri	high	PR	37	64

41	Bull terrier	m	11	22.8	PTCL	Vb	TBCT	pLN, tongue, larinx, ribs, muscle	low	wri	wri	wri	PD	0	55
42	Rottweiler	m	5	44.4	MZL	IVa	tRX, aUS	pLN, sleen, liver	wri	wri	wri	high	CR	58*	59§
43	Boxer	m	6	31.4	MZL	IVa	tRX, aUS	pLN, tLN, aLN, spleen, liver	low	wri	wri	high	CR	125*	126§
44	mixed	m	9	36.0	MZL	IVa	tRX, aUS	pLN, liver	wri	wri	wri	wri	PR	57	156
45	Poodle	m	8	13.5	MZL	IVa	tRX, aUS	pLN, tLN, spleen, liver	wri	wri	wri	high	PR	39	186§
46	Shih-tzu	m	5	8.8	MZL	IVa	tRX, aUS	pLN, spleen, liver	wri	low	wri	high	PD	0	33§
47	mixed	sf	14	24.4	MZL	IVb	tRX, aUS	pLN, tLN, spleen, liver	wri	wri	wri	high	PR	85	133
48	Poodle	f	14	11.2	MZL	IVb	tRX, aUS	pLN, tLN, aLN, spleen, liver	low	low	wri	high	PD	0	45
49	Dachshund	m	6	8.0	MZL	Va	TBCT	pLN, spleen, liver, PB	wri	wri	wri	wri	CR	99	208§

50	Jack Russell terrier	m	5	9.7	MZL	Vb	tRX, aUS	pLN, PB	wri	wri	wri	wri	CR	49	125
51	Basset Hound	m	7	31.1	MZL	Vb	tRX, aUS	pLN, spleen, liver, eye	wri	wri	wri	wri	PR	118	235
52	Beagle	m	11	20.6	T-SLL	Vb	tRX, aUS	pLN, spleen, PB, BM, skin	wri	wri	wri	wri	PR	110	111
53	Dalmatian	m	7	23.4	T-SLL	Vb	tRX, aUS	pLN, aLN, spleen, liver, PB, BM	wri	low	high	wri	PD	0	55
54	mixed	m	8	49.6	B-SLL	Vb	tRX, aUS	pLN, spleen, liver, PB, BM, eye	wri	low	wri	wri	PR	55	109
55	Boxer	f	4	28.9	LL	Vb	tRX, aUS	pLN, spleen, liver, PB	wri	low	wri	wri	PD	0	9
56	mixed	sf	11	38.3	LL	Vb	tRX, aUS	pLN, tLN, spleen, liver, PB, BM	wri	low	wri	wri	PD	0	13
57	Labrador retriever	m	2	30.5	LL	Vb	TBCT	pLN, tLN, BM	wri	low	wri	high	PD	0	5

58	Doberman pinscher dog	cm	6	36.8	FL	IVa	tRX, aUS	pLN, aLN, liver	wri	wri	wri	wri	CR	168	200
59	Yorkshire terrier	sf	15	4.3	FL	IVa	tRX, aUS	pLN, spleen, liver	wri	wri	wri	high	CR	46*	47§
60	American cocker spaniel	f	10	12.1	FL	IVa	tRX, aUS	pLN, aLN, spleen	low	low	wri	high	CR	157*	158§
61	Bernese Mountain dog	m	4	39.9	TZL	Va	tRX, aUS	pLN, spleen, liver, BM	wri	wri	wri	high	CR	271*	272§
62	schnauzer	sf	5	31.0	TZL	Vb	TBCT	pLN, tLN, aLN, BM	low	low	wri	wri	PD	21	28
63	English bulldog	cm	9	24.3	LPL	Vb	tRX, aUS	pLN, skin	wri	wri	wri	wri	CR	576*	577§

PCV = packed cell volume. PLT = platelet count. Ca = calcemia. LDH = total lactic dehydrogenase activity. RTT = response to therapy. TTP = time to progression. LSS = lymphoma specific survival. m = male. f = female. cm = castrated male. sf = spayed female. DLBCL = diffuse large B-cell lymphoma. PTCL = peripheral T-cell lymphoma. MZL = marginal zone lymphoma. B-SLL = B-cell small lymphocytic lymphoma. T-SLL = T-cell small lymphocytic lymphoma. LL = lymphoblastic lymphoma. FL = follicular lymphoma. TZL = T-zone lymphoma. LPL = lymphoplasmacytic lymphoma. tRX= thoracic radiographs. aUS=abdominal ultrasound. TBCT=total body CT. pLN= peripheral lymph nodes. tLN= thoracic lymph nodes. aLN=abdominal lymph nodes. PB=peripheral blood. BM=bone marrow. wri=within reference interval. CR=complete remission. PR=partial

673 remission. SD=stable disease. PD=progressive disease. ND=not determined. *=still in CR at data analysis closure. [§]=still alive at the end of the
674 study. °=dead for unrelated causes.

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679 Table 3. Response to therapy by histological subtype in 63 dogs with lymphoma.

	Number (%) of cases								
		Histological subtype							
Response	**No**	**DLBCL**	**PTCL**	**MZL**	**TZL**	**SLL**	**FL**	**LL**	**LPL**
CR	38	23(82.1)	6(46.2)	4(40)	1(50)	0	3(100)	0	1(100)
PR	10	1 (3.6)	3(23.1)	4(40)	0	2(66.7)	0	0	0
SD	2	1 (3.6)	1(7.7)	0	0	0	0	0	0
PD	10	1(3.6)	2(15.4)	2(20)	1(50)	1(33.3)	0	3(100.0)	0
nd	3	2 (7.1)	1 (7.7)	0	0	0	0	0	0
Total	63	28	13	10	2	3	3	3	1

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681

682 Table 4. Distribution by morphological group and clinical stage of 62 dogs with lymphoma.

Morphological group	Number (%) of cases			
	Clinical stage			
	III	IV	V	Total
Aggressive B-cell	5 (18.5)	17 (63)	5 (18.5)	27
Aggressive T-cell	4 (25)	2 (12.5)	10 (62.5)	16
Indolent B-cell	0	10 (66.7)	5 (33.3)	15
Indolent T-cell	0	0	4 (100.0)	4
Total	9	29	24	62

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684

685 Table 5. Median TTP and LSS by stage and morphological group in 63 dogs with
686 lymphoma.

	TTP (days)		LSS (days)	
	median	range	median	range
Stage III	245	0-423	NR	23-459
Aggressive B-cell	300	28-423	NR	28-424
Aggressive T-cell	47	0-315	53	23-459
Stage IV	134	0-457	200	13-544
Aggressive B-cell	137	19-457	200	13-544
Indolent B-cell	85	0-168	200	33-200
Aggressive T-cell	29	-	30	13-30
Stage V	70	0-576	111	5-577
Aggressive B-cell	192	34-240	256	71-530
Indolent B-cell	99	49-576	235	109-577
Aggressive T-cell	37	0-572	55	5-572
Indolent T-cell	21	0-271	55	28-272

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690 **Running headline**: Clinical spectrum of canine lymphoma

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692

693 Figure 1: Kaplan-Meier curves representing lymphoma-specific survival in 63 dogs,
694 divided into morphological groups on the basis of morphology and immunophenotype.
695 "•" = censored data.