Canine nodal marginal zone lymphoma: Descriptive insight into the biological behaviour

CANINE NODAL MARGINAL ZONE LYMPHOMA: DESCRIPTIVE INSIGHT INTO THE BIOLOGICAL BEHAVIOUR

Abstract

Canine nodal Marginal Zone Lymphoma (nMZL) is classified as an indolent lymphoma. Such lymphomas are typified by low mitotic rate and slow clinical progression. While the clinical behavior of canine splenic MZL has been described, characterized by an indolent course and a good prognosis following splenectomy, there are no studies specifically describing nMZL. The aim of this study was to describe the clinical features of and outcome for canine nMZL. Dogs with histologically-confirmed nMZL undergoing a complete staging work-up (including blood analysis, flow cytometry (FC) on lymph node (LN), peripheral blood and bone marrow, imaging, histology and immunohistochemistry on a surgically-removed peripheral LN) were retrospectively enrolled. Treatment consisted of chemotherapy or chemo-immunotherapy. Endpoints were response rate (RR), time to progression (TTP) and lymphoma-specific survival (LSS).

A total of 35 cases were enrolled. At diagnosis, all dogs showed generalized lymphadenopathy. One-third were systemically unwell. All dogs had stage V disease; one-third also had extranodal involvement. The LN population was mainly composed of medium-sized CD21+ cells with scant resident normal lymphocytes. Histology revealed diffuse LN involvement, referring to "late-stage" MZL. Median TTP and LSS were 149 and 259 days, respectively. Increased LDH activity and substage b were significantly associated with a shorter LSS.

Dogs with nMZL may show generalized lymphadenopathy and an advanced disease stage. Overall, the outcome is poor, despite the "indolent" designation. The best treatment option still needs to be defined.

Keywords: lymphoma, indolent lymphoma, clinical presentation, outcome, MZL, dog

INTRODUCTION

Canine Marginal Zone Lymphoma (MZL) is an indolent Non-Hodgkin lymphoma (NHL) that originates from the marginal zone of B-cell follicles. It has been described as an indolent disease, having a low mitotic index and a slow clinical progression.[1]

In human medicine, the MZL group is divided into 3 subtypes according to the World Health Organization (WHO) classification[2]: Mucosal Associated Lymphoid Tissue Lymphoma (MALT), splenic Marginal Zone Lymphoma (sMZL) and nodal Marginal Zone Lymphoma (nMZL). These three entities are described as separate diseases in terms of biology, clinical presentation and behavior. The major diagnostic criterion is the site of presentation.[3] MALT is relatively common, encompassing 5–8% of all NHLs and it most frequently involves the gastrointestinal tract (66% of all MALT cases), occurring in patients with a history of autoimmune disorders and chronic inflammation.[4,5] sMZL and nMZL are quite rare, each comprising less than 1% of NHL. sMZL is a symptomatic disease which at onset usually involves spleen, bone marrow (BM) and peripheral blood (PB), and is generally associated with splenomegaly and hematological alterations. Many patients with nMZL show regional (head and neck) lymphadenopathy, but more than 70% present with stage III/IV disease (according to the Ann Arbor staging scheme).[6,7]The prognosis is reported to be less favorable for nMZL than for MALT and sMZL.[8]

The WHO classification of hematopoietic and lymphoid tumors of domestic animals also divides canine MZL into the same three entities. In dogs, MALT lymphoma has not been well described. It is rare, and predominantly involves the respiratory and intestinal tracts, but other locations have been occasionally reported, such as the salivary gland and eyelid.[1,9] Canine sMZL has been described in terms of presentation and outcome. Unlike human sMZL, the majority of canine sMZL represents an incidental finding during physical examination and abdominal ultrasound. Canine sMZL has an indolent clinical course and splenectomy, with or without systemic chemotherapy, is usually curative.[10-12]

Canine nMZL is generally classified as an indolent lymphoma. However, in spite of the putative indolent nature, some dogs with nMZL may experience an aggressive disease course.[13-17] Specific studies focused on clinical presentation and behavior of canine nMZL are lacking.

The aim of the present study is to describe the clinico-pathological features and outcome in a cohort of dogs with histologically- confirmed MZL with a primary nodal presentation, thus better characterize this tumor as a single disease entity.

MATERIALS AND METHODS

Inclusion criteria

Medical records of dogs with lymphoma referred to the Centro Oncologico Veterinario between 2012 and 2016 were retrospectively reviewed for cases with a histopathological diagnosis of nMZL.[1,18]

To be eligible for enrolment, dogs were required to undergo a complete staging work-up, including complete blood count (CBC), serum biochemistry (including Lactate Dehydrogenase-LDH-activity and Ionized Calcium concentration), cytology and flow cytometric immunophenotyping on a lymph node (LN) aspirate, thoracic radiographs, abdominal ultrasound, fine-needle aspiration of liver and spleen regardless of their sonographic appearance, and PB and BM infiltration degree assessed by flow cytometry (FC). The abovementioned work-up is standard of care in the Centro Oncologico Veterinario. Previous lymphoma-directed therapy (including steroids) was not permitted.

Flow Cytometry

FC was performed on fresh samples of LN aspirates, collected into RPMI 1640 (Sigma Aldrich, St Louis, MO, USA) and processed as previously described.[19] Cells were investigated with a multicolor approach by FACScalibur cytometer (Becton Dickinson, San Jose-California USA), using antibodies against the following markers: CD45 (panleukocytes, clone YKIX716.13, Serotec, Oxford, UK), CD3 (T cells, clone CA17.2A12, Serotec, Oxford, UK), CD5 (T cells, clone YKIX322.3, Serotec, Oxford, UK), CD4 (T helper cells, clone YKIX302.9, Serotec, Oxford, UK), CD8 (T cytotoxic cells, clone YCATE55.9, Serotec, Oxford, UK), CD21 (mature B cells, clone CA21D6, Serotec, Oxford, UK), CD79a (B cells, clone MCA1298F, Serotec, Oxford, UK), CD34 (precursor cells, clone 1H6, Pharmigen, BD, Bioscience, USA) and MHC-II (antigen presenting cells, clone G46-6, Pharmigen, BD, Bioscience, USA). Cell viability was evaluated using Propidium Iodide (PI) and

considered adequate if >50% cells were PI-negative. Data analysis was performed with Cell Quest Pro software (Becton Dickinson, San Jose, California).

PB and BM involvement were defined as the presence of cells of B-lineage (CD21 positive) of medium to large size. Although specific cut-off values for defining tumor infiltration in PB and BM have not been defined for canine MZL, these were set at 0.56% for PB and 2.45% for BM, respectively, out of the total CD45 positive cells. These values were derived from a recent study on the analytical and diagnostic performances of FC to detect PB and BM neoplastic infiltration of large B-cell lymphoma cells in dogs.[20]

Cytological smears of LN, PB and BM aspirates were evaluated in parallel with FC in order to confirm cell morphology, evaluate mitotic figures and detect neoplastic infiltration.[21]

Histology

A peripheral enlarged LN was surgically removed, formalin-fixed and paraffin embedded, stained with haematoxylin and eosin, and examined by a veterinary pathologist (LA). For immunohistochemistry, antibodies against CD3 (clone F7.2.38; Dako), CD5 (clone CD5/54/F6; Dako), CD79a (clone HM57; Dako) and CD20 (clone RB-9013-P, Thermo Fisher Scientific) were used on paraffin-embedded sections. The diagnosis of nMZL was confirmed according to the WHO classification.[22]

Treatment and outcome

Dogs were treated with a 20-week combination induction chemotherapy, consisting of L-Asparaginase (week 1), vincristine (week 2, 3, 4, 13), cyclophosphamide (week 2, 13), doxorubicin (week 7, 16), lomustine (week 10, 19), and prednisone (week 1 through 20), as previously described.[17] Dogs whose owners wished to pursue immunotherapy, also received an intradermal injection of 0.5 ml autologous vaccine on weeks 4, 5, 6, 7, 12, 16, 20, and 24. The vaccines consisted of tumor-derived heat shock protein-peptide complex coupled with hydroxyapatite ceramic powder.[17,23] Response to treatment was classified as complete remission (CR), partial remission (PR), stable disease (SD) or progressive disease (PD)[24]. Response was evaluated at each therapeutic session and was required to last for at least 28 days.

Statistical Analysis

Time to progression (TTP) was calculated as the interval between initiation of treatment and PD or relapse, whereas lymphoma-specific survival (LSS) was measured as the interval between initiation of treatment and lymphoma-related death. Dogs lost to follow-up or dead for lymphoma-unrelated causes before PD, as well as those still in CR at the end of the study, were censored for TTP analysis. Dogs alive at the end of the study, lost to follow-up or dead due to causes other than lymphoma were censored for LSS analysis.

Response rate (RR) was defined as the sum of all dogs achieving CR and PR. Survival was analyzed according to the method of Kaplan-Meier. Differences between survival curves were evaluated with the log-rank test.

Multivariate analyses were performed using a Cox stepwise proportional hazard model to identify variables that might be of independent significance influencing TTP and LSS. Variables considered were: breed (mixed or pure), sex, age (cutoff arbitrarily set at 7 years), weight (cutoff arbitrarily set at 10 kg), PCV (normal, decreased, increased), platelet count (normal, decreased, increased), serum LDH activity (normal, decreased, increased), serum Ionized Calcium concentration (normal, decreased, increased), substage (a or b), spleen involvement (yes or no), PB infiltration (yes or no), total lymphocyte count in peripheral blood (as a continuous variable), BM infiltration (yes or no) and extranodal site involvement (yes or no).

Binomial logistic regression was performed to investigate the independence of LDH activity and response to treatment with respect to the abovementioned variables.

Statistical analysis was performed via SPSS v20.0 for Windows. Significance was set at $P \leq 0.05$ for all tests.

RESULTS

Thirty-five dogs met the inclusion criteria. Among them, 29 have been included in a previous paper.[17] Nine dogs (25.9%) were mixed breeds, while 26 (74.3%) were pure breeds (Table 1).

The median age was 7.0 years (mean 7.6±3.1 years, range 3.0-15.0 years). In particular, 15 dogs (42.9%) were younger than 7 years old, while 20 dogs (57.1%) were 7 or more years old. Median weight was 24.6 kg (mean 23.0±12.5 kg, range 3.0-44.4 kg), with 7 dogs (20%) less than 10 kg and 28 (80%) 10 kg or more. There were 21 (60%) males (3 neutered) and 14 (40%) females (5 spayed).

All dogs were presented with generalized lymphadenopathy and this was the reason for initial presentation. Lymphadenopathy had been present for a median of 20 days (range, 2-120 days). At the time of diagnosis, 23 (65.7%) cases were asymptomatic, while 12 (34.3%) showed non-specific clinical symptoms. All dogs had stage V disease. Splenomegaly was detected during physical examination in 20 (57.1%) dogs. However, the percentage of cases with splenic involvement rose up to 97.1% (34 dogs) based on abdominal ultrasound and cytological evaluation. In these dogs, abdominal ultrasound revealed splenomegaly; the spleen showed abnormal echogenicity and echo-structure, with a diffusely heterogeneous parenchyma. Splenomegaly was considered moderate in 30% and severe in 70% of the cases. Focal lesions were often observed (70% of the dogs), represented by 1-2 cm large hypoechoic nodules or multiple small hypoechoic nodules, with a consequent spotted appearance of the parenchyma ("honey-comb appearance"). The sonographic findings suggested parenchymal infiltration, confirmed by cytological evaluation showing a homogeneous or highly prevalent population of medium sized blast cells, often with macronucleoli. The liver was infiltrated in 27 (77.1%) dogs, as documented by sonographic changes and confirmative cytology. In addition, 10 (28.6%) dogs had extranodal involvement, with the lung present (9 cases), while only 1 dog had more than one extranodal site documented (eye and bladder). Lymphoma at extranodal sites was diagnosed by imaging and confirmative cytology in all but one cases; the dog with ocular involvement had a resolution of bilateral uveitis after the first chemotherapy administration, consistent with a presumptive neoplastic nature of the lesion.

Cytologically, the neoplastic cells were medium-sized and characterized by nuclei of intermediate size (1.5- 2x the size of a red blood cell) with fine chromatin, prominent single central nucleoli and a moderate amount of weakly basophilic cytoplasm. Few residual mature lymphoid cells were also present. Sometimes a scant population of larger lymphoid cells, defined as centroblasts with anisocytosis and anisokaryosis, was observed. Mitotic index was low with less than 1 mitotic figure/5 HPF (40x).

FC confirmed the B-cell lineage of the neoplastic cells. CD21 and/or CD79a positive cells represented the predominant cells in LN samples (median=82.7%, range 42.0-95.7, mean 78.1±15.6). They showed median FSC of 432.8 (mean 440.5 ± 46.2, range 357.1-521.6). An admixed population of small residual lymphocytes was also present, yet scarce in percentage (median=6.4%, mean 12.4±18.2%, range 3.0-22.8%). Regarding

PB and BM infiltration, 34 (97.1%) dogs had PB involvement, with a median percentage of neoplastic cells of 6.4% (mean 12.4±18.2%, range 0.7-53.5%), while BM was infiltrated in 20 (57.1%) cases, with a median percentage of neoplastic cells of 8.1% (mean 12.5±11.4%, range 3.0-51.6%).

Histology and immunohistochemistry were performed in all cases. Histological grade was available for 31 cases; among them, 30 were at a late stage of development, characterized by a diffuse growth pattern and loss of follicle-related architecture. The capsule was documented to be thinned and taut. The greatest proportion (80-90%) of cells was medium-sized (1.5-2x red blood cell), with scant eosinophilic cytoplasm, round nucleus and single prominent central nucleolus. The remaining 10-20% of the LN population was represented by small mature lymphocytes. Sometimes, large cells defined as centroblasts and immunoblasts were observed; mitotic activity of these cells was variably, low to moderate, and the mean mitotic index ranged from 0 to 5 in 10 HPF (40x).[16] Tingible body macrophages were present. CD79a and CD20 immunohistochemical positivity confirmed B-cell origin.[1,22]

Regarding CBC at diagnosis, 3 (8.6%) dogs were anemic (PCV<37%), 31 (88.5%) had a PCV within the reference interval, and 1 (2.9%) dog had an increased PCV (57%); 4 (11.4%) dogs had thrombocytopenia (platelet count <200 10^3/µL confirmed by smear evaluation), while 31 (88.5%) had a normal platelet count. Thirty-four dogs were normocalcemic, while one dog had a decreased ionized calcium concentration. LDH activity (< or ≥300 IU/L) was increased in 14 (40%) cases, while it was normal in the remaining 21 (60%) dogs. Binomial logistic regression revealed no significant correlation between LDH activity and all abovementioned variables. No correlation was found between PB lymphocyte count and TTP or LSS.

Treatment and outcome

Thirty-four dogs were treated with chemotherapy, and 18 (52.9%) received concurrent immunotherapy. One dog received no treatment at all and was excluded from the survival analysis. TTP and LSS for this dog were 49 and 340 days, respectively.

Of the 34 dogs that were treated, 25 completed the planned treatment schedule and 9 died during treatment due to PD. Among all others, 20 (80%) achieved CR (of those, 13 received concurrent immunotherapy) and 5 (20%) achieved PR (of those, 3 received concurrent immunotherapy).

Sixteen dogs having completed the planned protocol received rescue chemotherapy after documentation of PD: 12 received a CHOP-based protocol, whereas 3 were treated with DMAC (dexamethasone, d-actinomycin, melphalan, cytosine arabinoside). A second CR was obtained in 14 of them.

Binomial logistic regression revealed that platelet count was significantly associated with treatment response (p=0.033). Thrombocytopenic dogs had a lower probability of responding to treatment (OR=0.071; 95% CI 0.006-0.810). RR was 50% for dogs with thrombocytopenia and 80% for dogs with a normal platelet count.

Overall median TTP was 149 days (range 1-994 days). None of the investigated variables significantly influenced TTP.

Overall median LSS was 259 days (range 5-1605 days). Four dogs were alive at data analysis closure after 601, 613, 1016 and 1605 days. Three dogs died of tumor-unrelated causes after 93, 181 and 238 days. Cause of death was due to lymphoma in the remaining 28 dogs.

LDH activity (p=0.025) and substage (p=0.008) significantly influenced LSS. In particular, median LSS was 385 days (range 111-1605 days, n=20) for dogs with a normal LDH serum level, and 211 days (range 5-601 days, n=14) for dogs with increased LDH; asymptomatic dogs (substage a) had a median LSS of 399 days (range 93-1605, n=22), compared to 125 days (range 5-613, n=12) for symptomatic dogs (substage b). Multivariate Cox's proportional hazard regression analysis showed the influence of platelet count (p=0.01) on LSS.

DISCUSSION

This study describes the clinical presentation and outcome of 35 dogs with histologically-confirmed nMZL. Despite the retrospective nature of the design, data concerning initial staging, treatment and follow-up were available for all dogs, thereby providing robust information. Canine nMZL is considered an indolent disease, occurring in adult dogs that usually retain normal appetite and activity, and could be characterized by the enlargement of a single lymph node, typically in the submandibular or cervical lymph node chain,[22] or by a generalized lymphadenopathy.[16]

However, the published studies describing the clinical and morphological features of indolent lymphomas suggest that a subset of nMZL cases may display a more aggressive clinical course.[13-17] Indeed, based on our results, the indolent designation may not always be appropriate, as all dogs had generalized lymphadenopathy and one third of them were symptomatic at initial presentation. A complete staging work-up for all cases showed that most dogs had an advanced disease stage at onset.

In contrast with previous reports, suggesting that nMZL typically does not cause any systemic involvement,[1] all dogs but one had PB involvement and 57.1% had BM involvement, while one third of them had extranodal involvement. The cause for the discrepancy between PB and BM infiltration is unclear, but an overspill of neoplastic cells from affected nodes in the absence of true BM invasion could be a possible explanation, similar to what has been described for T-zone lymphoma.[26] Alternatively, it may be due to the different cut-offs used to define positive PB and BM samples and to the use of FC for staging, which is a very sensitive tool to detect BM and PB infiltration compared with standard light microscopy.[21]

Although splenic involvement was detected in 97% of cases, a primary splenic MZL was considered unlikely, based on the integration of clinical and pathological data. Indeed, in canine primary sMZL, the spleen is usually the only site involved, and the diagnosis is frequently incidental.[10,11,13] In the present study, ultrasonographic findings including splenomegaly, diffuse heterogeneity and hypoechoic nodular lesions suggested diffuse secondary infiltration of the parenchyma. Conversely, primary splenic MZL is characterized by a solitary focal hypoechoic mass without any changes of the surrounding tissue.[10,11,22]

In humans, sMZL usually also involves liver, BM and PB, and is complicated by anemia and thrombocytopenia. Peripheral lymphadenopathy is infrequent (15–25% of cases), but splenic hilar LNs may be involved (35–65% of cases);[27] nMZL is defined by the WHO classification as "a primary nodal B-cell neoplasm that morphologically resembles LNs involved by MZL of extranodal or splenic types, but without evidence of extranodal or splenic disease". This implies that also in humans the diagnosis of nMZL is mainly based on the pattern of dissemination of the disease, essentially based on the fact that sMZL involves the spleen without concomitant peripheral lymphadenopathy, while nMZL does not have a clinical evidence of extranodal or splenic disease.[2,28]

In spite of these considerations, we cannot definitely rule out a primary splenic origin of the tumor with a secondary late dissemination to peripheral nodes. In human medicine, progress in the field of molecular biology has aided differentiation between nMZL, sMZL and MALT. A molecular and cytogenetic variability between the three subtypes has emerged, but to date no unique alterations have been documented.[29] In particular, among human B-cell neoplasms, nMZL is still lacking specific genetic lesions, although recently the occurrence of a gene deletion involving the receptor-type tyrosine-protein phosphatase delta (PTPRD) was found in a cohort of nMZL cases.[30]

For the cases included in the present study, we were able to evaluate the neoplastic population by means of three different techniques, namely cytology, histopathology and FC. The different techniques gave concordant and overlapping information: samples mainly comprised medium-sized cells, but sometimes were accompanied by a population of centroblasts/immunoblasts and scant resident small lymphocytes. These features also correspond to those previously described in the literature for humans, where the presence of sheets of centroblasts appears to be related to disease progression and tumor transformation into large B-cell lymphoma.[31] Indeed, histology revealed that all cases but one were at a late stage of development, characterized by a diffuse growth pattern and loss of the follicle-related architecture. The only dog with the classical histologic marginal presentation and a slight PB and BM infiltration experienced long LSS (680 days). More cases are needed to define if the histological architecture pattern may be independently associated with a differing clinical behavior.

Based on the above, it may be hypothesized that late-stage nMZL behaves clinically like high-grade lymphomas, with a tendency to spread systemically. Accordingly, Richards et al. found molecular similarities between nMZL and diffuse large B-cell lymphoma (DLBCL), suggesting that these conditions might represent a continuous spectrum of the same disease.[32] Although tumor transformation into a more aggressive disease has only been rarely reported in veterinary oncology,[33] an evolution from nMZL into DLBCL may be hypothesized from the available data.

Approximately one third of dogs died due to lymphomas within 6 months despite treatment, thereby exhibiting a poor outcome that contrasts with the "indolent" tumor designation. This discrepancy is likely due to the different inclusion criteria among studies. Equally, the case selection of the current study might

be biased as dogs with generalized lymphadenopathy are more likely to be referred to a referral center and undergo a full staging work-up. Indeed, this is the first case series focused exclusively on nMZL, with strict staging criteria. Most of the studies published in the veterinary literature include many different lymphoma subtypes or are limited to small case series with incomplete staging and follow-up data.[13-15]

Overall TTP and LSS were disappointingly low, suggesting that the CHOP-based protocol used in the current series of dogs may not be the best option. This may be due to the relatively low dose-intensity of the adopted protocol, and it remains to be elucidated whether a different chemotherapy dosing intensity may improve clinical outcome. Alternatively, the incorporation of different alkylating drugs may better target MZL cells.

Regarding prognostic factors, platelet count significantly influenced RR. Thrombocytopenic dogs had a significantly lower RR and shorter LSS. Thrombocytopenia is reported in 10-13% of cancer-bearing dogs,[34,35] and is generally considered to be a poor prognostic factor.[1,36,37] Substage b was an additional independent risk factor, in agreement with previous studies, showing a correlation with a poor outcome.[38,39]

Increased LDH serum level was also significantly associated with a shorter LSS. An increased LSH level at diagnosis has been associated with a shorter survival in people with indolent lymphoma.[40,41] It may be possible that the same holds true in dogs.

Surprisingly, PB and BM involvement were not significantly associated with outcome. This is in contrast with what has been described for DLBCL.[42] The cut-off values may have influenced the definition of stage. Indeed, in the present series all dogs had stage V disease when the currently defined cut-off values were applied, and a significant difference may not have emerged. It must be acknowledged that the cut-off values used in the current study and extrapolated from previously published data may be inappropriate for MZL, due to the smaller size of neoplastic cells that impede discrimination between neoplastic and reactive B-lymphocytes. A specific validation study is needed to define the correct FC approach for staging MZL.[20] The main limitation of the present study is the absence of cases with nodular presentation and earlier disease stages (I to IV). This prevents extrapolation about the clinical course of nMZL and the clinical significance of PB and BM involvement. As all dogs enrolled in the present study had generalized

lymphadenopathy, it is possible that dogs were initially asymptomatic for a long time and during that time regional lymphadenopathy may have gone unnoticed.

In conclusion, dogs with nMZL may present at an advanced stage of disease, with an overall poor prognosis despite the indolent designation. Due to the significant clinical interest, the issue of dose-intensity should be further explored in dogs with nMZL.

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Table 1. Clinical presentation and outcome of 35 dogs with nodal Marginal zone lymphoma

Breed	Sex	Age (years)	Thrombocytopenia	Stage	Substage	Immunotherapy	Response	Cause of death (days)
Akita inu	NM	9	no	V	b	yes	CR	Alive (613)
Australian shepherd	F	12	no	V	a	no	CR	Lymphoma (337)
Australian shepherd	M	11	yes	V	b	no	PD	Lymphoma (5)
Bassethound	M	7	no	V	b	yes	PR	Other (238)
Beagle	M	5	no	V	a	yes	CR	Alive (1605)
Bernese mountain dog	M	3	no	V	a	no	PR	Lymphoma (35)
Border collie	M	4	no	V	b	no	PR	Lymphoma (340)
Boxer	M	6	no	V	a	yes	CR	Lymphoma (544)
Dachshund	M	6	no	V	a	no	CR	Lymphoma (1042)
French bulldog	F	5	no	V	b	yes	CR	Lymphoma (215)
German shepherd	F	8	no	V	a	yes	CR	Other (93)
German shepherd	F	3	yes	V	a	no	CR	Lymphoma (127)
Golden retriever	M	4	no	V	a	yes	CR	Lymphoma (385)
Jack russel	M	5	no	V	b	yes	CR	Lymphoma (125)
Jack russel	M	7	no	V	a	no	CR	Lymphoma (730)
Labrador retriever	M	3	no	V	b	yes	CR	Lymphoma (259)
Labrador retriever	F	7	no	V	a	no	PR	Alive (1016)
Mixed	SF	15	no	V	a	no	PR	Lymphoma (133)
Mixed	M	9	no	V	a	yes	PR	Lymphoma (156)
Mixed	SF	6	no	V	a	yes	CR	Lymphoma (680)
Mixed	M	9	no	V	a	no	CR	Lymphoma (111)
Mixed	F	5	no	V	b	yes	CR	Lymphoma (188)
Mixed	M	9	no	V	b	yes	CR	Lymphoma (248)
Mixed	NM	9	no	V	a	no	CR	Lymphoma (632)
Mixed	F	9	no	V	b	no	PD	Lymphoma (20)
Mixed	SF	9	yes	V	a	yes	CR	Alive (601)

Petit bleu	SF	11	no	V	a	yes	CR	Other (181)
Pinscher	SF	11	yes	V	b	no	PD	Lymphoma (7)
Pomeranian	M	13	no	V	a	no	CR	Lymphoma (211)
Poodle	M	7	no	V	a	yes	PR	Lymphoma (399)
Poodle	F	14	no	V	b	no	PD	Lymphoma (45)
Rottweiler	M	5	no	V	a	yes	CR	Lymphoma (349)
Rottweiler	F	6	no	V	a	no	CR	Lymphoma (160)
Shih-tzu	M	5	no	V	a	yes	CR	Lymphoma (152)
Yorkshire terrier	NM	8	no	V	a	no	CR	Lymphoma(1403)

F, female; M, male; SF, ~~spayed female; NM, neutered male; Lymphoma, dead of lymphoma related causes; Other, dead of other causes~~ then lymphoma; CR, complete remission; PR, partial remission; PD, progressive disease.