Prognostic significance of peripheral blood and bone marrow infiltration in newly-diagnosed canine nodal marginal zone lymphoma

Original article

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Abstract

Canine nodal marginal zone lymphoma (nMZL) is infrequent and is typically diagnosed at an advanced disease stage. However, it is currently unknown whether different levels of peripheral blood (PB) and bone marrow (BM) infiltration may provide prognostic stratification in dogs with nMZL.

The aims of the present prospective study were to assess the influence of PB and BM infiltration detected by flow cytometry (FC) on time to progression (TTP) and lymphoma-specific survival (LSS) in dogs with newly-diagnosed multicentric nMZL, and to establish a cut-off value of prognostic significance.

Forty-five completely staged and treatment-naïf dogs with histologically-confirmed nMZL were enrolled. After staging, dogs received chemo-immunotherapy or chemotherapy. PB infiltration was significantly associated with TTP ($p=0.001$): dogs with PB infiltration <30% had a median TTP of 186 days, whereas dogs with PB infiltration ≥30% had a median TTP of 43 days. Additionally, vaccinated dogs had a significantly ($p=0.012$) longer TTP (399 days) compared with dogs receiving chemotherapy only (211 days).

BM infiltration was significantly associated with LSS ($p<0.001$): dogs with BM infiltration <1% had a median LSS of 1403 days, those with BM infiltration 1-20% of 337 days, and those with BM infiltration ≥20% of 188 days. Normal LDH levels and the administration of chemo-immunotherapy also significantly improved LSS (560 vs 211 days, and 399 vs 211 days, respectively; $p<0.001$).

PB and BM flow cytometric evaluation is an integral part of staging work-up in dogs with nMZL and has prognostic relevance.

Keywords: Dog; Bone marrow; Cut-off; Flow cytometry; Marginal zone lymphoma

Introduction

In dogs, nodal marginal zone lymphoma (nMZL) is infrequent, representing approximately 10% of all lymphoma histotypes diagnosed in this species (Valli et al., 2011). It was recently documented that nMZL is characterized by generalized lymphadenopathy and is diagnosed at an advanced disease stage; thus, despite the indolent designation, the prognosis is guarded (Cozzi et al., 2018).

Adequate management of canine lymphoma requires accurate histological diagnosis and comprehensive staging, which includes assessment of bone marrow (BM) involvement. Therapeutic options rely mainly on the results of these procedures (Marconato et al., 2017).

In dogs with diffuse large B-cell lymphoma (DLBCL), it has been shown that 3% BM infiltration evaluated by flow cytometry (FC) identified cases with an unfavorable prognosis (Marconato et al., 2013). According to a recent study, peripheral blood (PB) and BM infiltration occurred in 97.1% and 57.1% of canine nMZL cases, respectively (Cozzi et al., 2018). However, it is currently unknown whether different levels of PB and BM infiltration may provide prognostic stratification in dogs with nMZL.

The aims of the present prospective study were to assess the influence of different levels of PB and BM infiltration, detected by FC, on the duration of the first remission and survival in dogs with newly-diagnosed multicentric nMZL, and to establish a cut-off value of prognostic significance.

Material and methods

Inclusion criteria

A prospective analysis of dogs with multicentric nMZL was performed. To be enrolled in the study, dogs were required to have complete clinicopathological data for analysis, and be

73 treatment-naïf. Corticosteroids before admission were permitted.

74

75 All dogs were staged according to the World Health Organization system (Owen,
76 1980), comprising history, physical examination, haematology, serum biochemistry
77 (including serum lactate dehydrogenase, LDH), thoracic radiographs, abdominal ultrasound,
78 and cytological evaluation of a fine-needle aspirate of an enlarged peripheral lymph node
79 (LN). Dogs also underwent LN, PB and BM sampling for FC evaluation, and
80 lymphadenectomy of an enlarged peripheral LN, having histopathological evaluation and
81 immunohistochemistry (CD3, CD20) as a part of their initial staging work-up (Aresu et al,
82 2015). The same LN that was aspirated for obtaining a cytological diagnosis and for FC was
83 then surgically removed. The diagnosis of nMZL late-stage was confirmed according to the
84 WHO classification (Valli et al., 2011).
85 For the specific aims of the present research, at the end of the study, FC data were
86 blindly re-analyzed to minimize possible interpretation biases among different readers over
87 time. Only the re-analyzed results have been considered for the study.

88

89 The care of the dogs enrolled in the study was in accordance with institutional
90 guidelines. All owners provided written informed consent.

91

92 *Flow cytometry*

93 Flow cytometric immunophenotyping was performed on LN aspirates obtained with
94 22-gauge needles and collected in tubes containing RPMI1640 (Sigma Aldrich) and on PB
95 and BM samples collected into EDTA tubes. All samples were kept refrigerated and
96 processed within 24h of sampling. A panel of antibodies was used for LN labeling using a
97 multi-colour approach as previously described (Gelain et al., 2008), and included: CD45
98 (clone YKIX716.13, pan-leukocyte), CD5 (clone YKIX322.3, T-cells), CD21 (clone

CA2.1D6, B-cells), and CD34 (clone 1H6, precursor cells). PB and BM samples were stained with CD45, CD5 and CD21 antibodies. All antibodies but CD34 were provided by Bio-Rad (formerly AbD Serotec; Oxford, UK); CD34 was provided by BD Pharmingen (San Diego, CA, USA). Erythrocyte lysis was not necessary on LN samples, since haemodilution was minimal, and double labeling using CD45 easily enabled the distinction between lymphoid cells and debris or erythrocytes. Conversely, erythrocyte lysis was performed on PB and BM samples by means of an erythrocyte lysis buffer containing 8% ammonium chloride, after incubation with antibodies. All samples were acquired with a BD FACScalibur flow cytometer (Becton Dickinson, San Josè, CA, USA) and analyzed with CellQuest software (Becton Dickinson).

Determination of PB and BM involvement

PB and BM aspirates were obtained in all cases at the time of the initial staging work-up. BM was sampled with 16- or 18-gauge Illinois needles from the iliac crest. PB and BM samples were placed in EDTA tubes for FC analysis.

The extent of PB and BM infiltration by large B-cells was reported as the percentage of medium-large CD21-positive cells out of the total CD45-positive cells (leucocytes and their precursors). The threshold for cell size was set based on the FSC of non-neoplastic T-lymphocytes from the same subject. Normal circulating B-cells show the lowest FSC among lymphocytes, being smaller than normal T-cells. Reactive medium-sized B-cells present FSC properties partially overlapping those of T-cells. Thus, most of reactive medium-sized B-cells were not included in the count of infiltrating neoplastic cells with our gating strategy.

Treatment and response evaluation

The treatment protocol was in keeping with approved standards. Dogs whose owners wished to pursue immunotherapy received a 20-week dose-intense chemotherapy regimen,

consisting of L-Asparaginase, Vincristine, Cyclophosphamide, Doxorubicin, Lomustine, and prednisone (Table 1). These dogs also received an intradermal injection of 0.5 ml autologous vaccine on weeks 4, 5, 6, 7, 12, 16, 20, and 24, as previously described (Marconato et al., 2014).

Dogs treated with chemotherapy only, received the following protocol, consisting of L-Asparaginase, Vincristine, Cyclophosphamide, Doxorubicin, Lomustine, and Prednisone (Table 1).

Response was classified as complete remission (CR), partial remission (PR), stable disease (SD), or progressive disease (PD) based on previously published criteria (Marconato et al., 2013). Responses were required to last for ≥ 28 days.

Relapse was defined as clinical reappearance and cytological evidence of lymphoma in any anatomical site in dogs having experienced CR, whereas relapse for animals with PR was defined as progression.

Response was evaluated at each chemotherapy session by measurement of peripheral LNs. End-staging was carried out at the end of treatment, and every clinical, radiological, ultrasonographic, or laboratory investigation that disclosed abnormalities at pre-treatment staging was repeated. BM and PB were re-evaluated in all cases by FC. The end-staging results were necessary to assess treatment response.

Statistical analysis

Time to progression (TTP) was calculated from the start of treatment to disease progression (Vail et al., 2010). Dogs lost to follow-up or dead for lymphoma-unrelated causes before disease progression, as well as those in CR at the end of the study, were censored for TTP analysis.

Lymphoma-specific survival (LSS) was measured as the interval between the start of

151 treatment and death for lymphoma (Vail et al., 2010). Dogs alive at the end of the study, lost
152 to follow-up or dead due to causes other than lymphoma were censored for LSS analysis.
153
154 To detect possible associations between TTP and LSS, and PB and BM infiltration,
155 cases were subdivided into two groups based on the arbitrarily selected infiltration cut-offs of
156 1.0%, 3.0%, 5.0%, 10.0%, 20.0% and 30.0%. Thereafter, Kaplan-Meier curves were drawn
157 for each cut-off and compared using the log-rank test. Based on data distribution, the 30.0%
158 cut-off was not tested on BM samples. As two different BM cut-offs gave significant results
159 for LSS analysis, these were coupled to stratify the study population into three groups, which
160 were further tested by Kaplan-Meier curves and log-rank test.
161
162 Univariate Cox's proportional hazard regression analysis was performed to determine
163 a possible association between selected variables and TTP and LSS, respectively. Variables
164 with $p \leq 0.3$ at univariate analysis were then included in a backward elimination multivariate
165 analysis. For categorical variables, Kaplan-Meier curves were drawn and compared by log-
166 rank test.
167
168 The independent variables included in the analyses were: breed (pure or mixed), age
169 (< or ≥ 8 years), sex (male or female), weight (< or ≥ 22.5 kg), clinical stage (I-V), substage
170 (a or b), anemia (presence or absence), thrombocytopenia (presence or absence), LDH (within
171 or outside the reference interval [0-170 U/I]), FC PB infiltration (%), PB infiltration group
172 (<30% or ≥30%), FC BM infiltration (%), BM infiltration group (<1%, 1%-20%, or ≥20%),
173 therapy (chemotherapy or chemo-immunotherapy).
174 Possible differences in the aforementioned variables between the two treatment groups
175 were investigated with Pearson chi-squared test (for categorical variables) or with Mann-
176 Whitney test (for continuous, non-parametric variables).

All analyses were performed with a standard software (SPSS v20.0 for Windows), and significance was set at $p \leq 0.05$ for all analyses.

Results

Between 2011 and 2018, 45 cases met the inclusion criteria and were enrolled. There were 12 (26.7%) mixed-bred and 33 (73.3%) pure-breed dogs. Among these, there were 3 Rottweiler, 3 Poodle, 2 jack russel terrier, 2 Australian shepherd, 2 Pomeranian, 2 Golden retriever, 2 German shepherd, 2 French Bulldog, 2 Labrador retriever, and one each of the following: Bassethound, dachshund, shih-tzu, beagle, Petit Bleu, Yorkshire terrier, Border collie, Bernese Mountain dog, Pinscher, Akita Inu, Dobermann, Boxer, and German Hound.

Twenty (44.4%) dogs were females (11 spayed) and 25 (55.6%) were males (3 neutered). Mean age was 7.9±3.2 years (median 8 years, range 3-15 years), with 21 (46.7%) dogs being <8 year-old and 24 (53.3%) ≥8 year-old. Mean body weight was 22.5±12.5 kg (median 24.4 kg, range 3.0-44.4 kg); the body weight was <22.5 kg in 21 (46.7%) dogs and ≥22.5 kg in 24 (53.3%).

One (2.2%) dog had stage IIIa disease, 6 (13.3%) had stage IV disease (4 substage a, 2 substage b), and 38 (84.4%) had stage V disease (24 substage a, 14 substage b). Anemia and thrombocytopenia were present in 4 (8.9%) and 6 (13.3%) dogs, respectively. LDH activity was tested in 41 dogs and was increased in 17 (41.5%).

Mean PB infiltration at diagnosis was 10.43±12.82% (median 5.0%, range 0.2-53.5%). It was <1% in 8 (17.8%) dogs, <3% in 15 (33.3%), <5% in 20 (44.4%), <10% in 32 (31.1%), <20% in 36 (80.0%), <30% in 41 (91.1%), and ≥30% in 4 (8.9%).

Mean BM infiltration at diagnosis was 6.86±9.39% (median 3.0%, range 0.2-51.6%). It was <1% in 9 (20.0%) dogs, <3% in 22 (48.9%), <5% in 25 (55.6%), <10% in 36 (80.0%),

<20% in 42 (93.3%), <30% in 44 (97.8%), and ≥30% in 1 (2.2%).

Twenty-four (53.3%) dogs received chemo-immunotherapy, and 21 (46.7%) were treated with chemotherapy. Twenty (83.3%) of 24 dogs treated with chemo-immunotherapy and 13 (61.9%) of 21 dogs treated with chemotherapy achieved CR, confirmed by a complete end-staging. However, 17 (85%) of the 20 dogs treated with chemo-immunotherapy and all (100%) dogs treated with chemotherapy eventually relapsed.

The two treatment cohorts were homogeneous for all investigated variables.

Median TTP was 179 days (range, 1-1295 days). In particular, 40 (88.9%) dogs progressed during the study period, whereas 4 (8.9%) died for lymphoma-unrelated causes after 93, 151, 181 and 237 days, respectively, and one (2.2%) dog was still alive and in CR after 1295 days.

Median LSS was 337 days (range, 5-1403 days). In particular, 33 (73.3%) dogs died for their lymphoma during the study period, 8 (17.8%) were still alive at data analysis closure with a median follow-up of 701 days (range 281-1295 days), and 4 (8.9%) died for lymphoma-unrelated causes after 93, 151, 181 and 237 days, respectively.

The results obtained for the different PB and BM infiltration cut-offs are shown in Table 2. According to these results, cases were subdivided into two PB infiltration groups (<30% and ≥30%) and into three BM infiltration groups (<1%, 1-20%, and ≥20%).

Concerning TTP, univariate Cox's analysis and log-rank test gave significant results for substage (p=0.020 and p=0.017, respectively), treatment (p=0.037 and p=0.033, respectively) and PB infiltration groups (p=0.006 and p=0.002, respectively) (Fig 1). Multivariate analysis showed significant results for treatment (p=0.012) and PB infiltration

229 groups (p=0.001).

230 Median TTP for significant variables is shown in Table 3.

231

232 Concerning LSS, univariate Cox's analysis and log-rank test gave significant results
233 for substage (p=0.002 and p=0.001, respectively), LDH (p=0.050 and p=0.044, respectively),
234 PB infiltration groups (p=0.015 and p=0.008, respectively) and BM infiltration groups
235 (p=0.035 and p=0.022, respectively) (Fig 2). Multivariate analysis gave significant results for
236 LDH (p<0.001), treatment (p=0.002) and BM infiltration groups (p<0.001). In particular,
237 dogs with BM infiltration <1% had a median LSS of 1403 days, whereas dogs with BM
238 infiltration of 1-20% and ≥20% had a median LSS of 337 and 188 days, respectively.

239 Median LSS for significant variables is shown in Table 4.

240

241 **Discussion**

242 Recent advances in imaging and laboratory methodology have the potential to improve
243 disease characterization and outcome in dogs with lymphoma, possibly leading to changes in
244 clinical practice and, eventually, in trial design. PB and BM evaluation is part of the staging
245 work-up for canine lymphoma, and FC is essential to detect and quantify their involvement
246 (Riondato et al., 2016; Riondato et al., 2017). However, knowledge about the prognostic value
247 of PB and BM infiltration based on FC in canine nMZL is limited.

248 The results of the current study suggest, in a homogeneous series of 45 dogs with
249 newly-diagnosed nMZL late-stage, that PB and BM infiltration detected by FC at diagnosis is
250 frequent and influences the duration of first remission and survival.

251 PB involvement in nMZL as detected by FC significantly influenced TTP. The
252 presence of circulating neoplastic cells was related to clinical stage and BM involvement, and
253 correlated with poor disease control. Indeed, dogs with <30% PB involvement had a median
254 TTP of 186 days, whereas dogs with ≥30% PB involvement relapsed earlier (TTP 43 days).

Lymphoma cells in PB reflect the biological property of entry of solid tumor cells into the circulation. Relapse is due either to incomplete remission in dogs with extensive tumor burden or to chemoresistance. Dogs with marked (≥30%) PB involvement had a very short remission; therefore, it may be possible that these cells were chemoresistant or had temporarily resided at sites poorly accessible to chemotherapy.

It has been previously documented that indolent B-cell lymphoma appears to be associated with a higher incidence of BM infiltration compared with aggressive B-cell lymphoma (33-80% versus 18-27%, respectively) (Aresu et al, 2015; Marconato et al., 2015a, Cozzi et al., 2018), thereby confirming the need for extensive staging in all lymphoma cases. Indeed, dogs with nMZL showed a heterogeneous clinical course based on BM infiltration, which resulted to be an independent predictor for LSS. Dogs with BM infiltration <1% had a significantly longer survival time compared with dogs with BM infiltration in the range of 1-20% and ≥20%. Moreover, despite the overall poor treatment outcomes in nMZL cases with BM infiltration, dogs with less extent of BM infiltration (1-20%) had a more favorable outcome compared with dogs in the other group (12 months versus 6 months, respectively), stressing the need not only for the detection of marrow involvement, but also for neoplastic cell quantification.

Beside PB and BM involvement, further variables were independent predictive factors in multivariate analysis.

An elevated LDH activity at initial diagnosis was predictive for shorter survival. This is in agreement with a previous study, whereby it was shown that elevated LDH activity was more frequent in dogs with DLBCL and higher PB and BM infiltration, possibly indicating a marked disease activity. Also, in the same study, high LDH activity predicted a more aggressive clinical course (Marconato et al., 2013). The same may hold true for nMZL.

Dogs receiving chemo-immunotherapy had a significantly longer TTP and LSS compared with dogs treated with chemotherapy only. In a previous study (Marconato et al.,

2014), chemo-immunotherapy significantly improved TTP in dogs with indolent B-cell lymphoma when compared to dogs receiving chemotherapy only, whereas LSS only tended to be longer. The differences between these studies might be explained by sample size and the different enrollment criteria, because the earlier study included all subtypes of indolent lymphoma as one entity instead of individual subtypes.

Interestingly, when comparing BM infiltration data with the ones previously published in DLBCL (Marconato et al., 2013), a better stratification for TTP and LSS was obtained in dogs with nMZL. This result is also emphasized by the diagnostic and biological limits that have been described so far for these two histotypes. Indeed, the histological diagnosis of nMZL late-stage is still a conundrum because of the lack of immunohistochemical markers that allow in many cases distinguishing it from DLBCL. To rule out this risk, we included only nMZLs that were histologically characterized by a diffuse infiltration of neoplastic cells with intermediate-sized nuclei, prominent single central nucleoli, abundant lightly stained cytoplasm, low mitotic rate and absence of centroblasts and immunoblasts. Moreover, two gene expression profile studies have recently shown that nMZL and DLBCL are similar at the transcriptomic level (Frantz et al., 2013), but they result two separate entities clinically, suggesting other biological mechanisms underlying tumor cells of origin.

This study has some limitations.

First, there was treatment regimens heterogeneity in the population of our study. Approximately 50% of dogs received an autologous vaccine in addition to conventional chemotherapy. Analyses of prognostic factors for TTP and LSS showed a better outcome for dogs that received chemo-immunotherapy. As of today, there are no standard recommended treatments for nMZL, which has not proven curable with classical chemotherapy (Aresu et al, 2015, Marconato et al., 2015b). In the current study, the combination of chemotherapy and immunotherapy significantly improved outcome. This finding raises the question as to possible choices in terms of classical chemotherapy and whether immunotherapy should be

the new gold standard for first-line treatment in dogs with nMZL.

Second, the FC strategy used to quantify PB and BM infiltration in the present study has never been validated for canine nMZL, but was derived by the one used to stage canine large B-cell lymphomas (Riondato et al., 2016). In particular, we lowered the FSC threshold in order to include medium-sized cells in the count. This likely affects the analytical and diagnostic performances reported. In particular, the limit of detection (LOD) of medium-large CD21+ cells in non-neoplastic samples is likely higher than the one reported for large B-cells, and even higher than 1%. Nevertheless, we report relevant prognostic implications only for highly infiltrated BM samples (>20%), which is likely far from the LOD of the technique, and for samples with <1% infiltration, where neoplastic cells are virtually absent. Cases potentially located between 1% and LOD would fall in the limbo of the 1-20% BM infiltration group, which bears an intermediate prognosis and warrants examination of further prognostic parameters.

Conclusions

The detection of PB and BM involvement in most dogs with nMZL confirms the need for PB and BM flow cytometric evaluation as an integral part of staging work-up also in these patients. We found that PB infiltration ≥30% and BM infiltration ≥20% in dogs with nMZL are independent negative prognostic factors. More specifically, in dogs with PB infiltration <30%, BM evaluation stratifies dogs into 3 prognostic groups: those with a poor (infiltration ≥20%), intermediate (infiltration 1-20%) and better (infiltration <1%) prognosis.

Future efforts should be directed toward finding optimal treatment modalities in managing nMZL dogs based on the extent of PB and BM infiltration.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Table 1

Therapeutic protocols administered to 45 dogs with Marginal Zone Lymphoma (MZL).

	Week number																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Chemo-immunotherapy protocol																			
L-ASP	X																		
VCR		X	X	X									X						
CYCLO		X											X						
DOXO							X									X			
CCNU										X									X
Prednisolone administered at 1 mg/kg daily from week 1 to week 4; then 0.5 mg/kg daily until the end of treatment																			
Chemotherapy protocol																			
L-ASP	X																		
VCR		X			X						X						X		
CYCLO			X			X						X						X	
DOXO				X			X						X						X
CCNU									X						X				
Prednisolone administered at 1 mg/kg daily from week 1 to week 4; then 0.5 mg/kg daily until the end of treatment																			

L-ASP = L-asparaginase (400 UI/kg SQ); VCR = Vincristine (0.75 mg/m2 IV); CYCLO = Cyclophosphamide (250 mg/m2 PO in the chemo-immunotherapy protocol, 75 mg/m2 PO for 4 consecutive days in the chemotherapy protocol); DOXO = Doxorubicin (30 mg/m2 IV); CCNU = Lomustine (80 mg/m2 PO)

Table 2

P-values obtained by comparing Kaplan-Meier curves using different arbitrarily selected cut-offs for peripheral blood and bone marrow infiltration in 45 dogs diagnosed with Marginal Zone Lymphoma.

Matrix	Cut-off	Log-rank test p-value	
		Time to progression	Lymphoma specific survival
Peripheral blood	1%	0.416	0.419
	3%	0.515	0.614
	5%	0.315	0.463
	10%	0.510	0.824
	20%	0.183	0.572
	30%§	0.002*	0.008*
Bone marrow	1%	0.098	0.039*
	3%	0.155	0.102
	5%	0.241	0.258
	10%	0.188	0.237
	20%	0.092	0.044*
	1% and 20%§	0.077	0.022*

*= significant result; §= cutoff selected for further survival analyses

Table 3

Time to progression (TTP) in 45 dogs with nodal Marginal Zone Lymphoma, according to specific variables

Variable (number of dogs)	Median TTP in days (range)	P-value Univariate analysis	P-value Log-rank test	P-value Multivariate analysis	Hazard ratio (95% CI)
Substage		0.020*	0.017*	0.930	
a (29)	312 (24-1295)				Ref
b (16)	60 (1-420)				2.195 (1.131-4.263)
Therapy		0.037*	0.033*	0.012*	
Chemotherapy (21)	78 (1-1295)				1.950 (1.040-3.658)
Chemo-immunotherapy (24)	227 (29-720)				Ref
PB infiltration		0.006*	0.002*	0.001*	
<30% (41)	186 (1-1295)				Ref
≥30% (4)	43 (1-134)				4.897 (1.579-15.188)

*=significant result

Table 4

Lymphoma specific survival (LSS) in 45 dogs with nodal Marginal Zone Lymphoma, according to specific variables.

Variable (number of dogs)	Median LSS in days (range)	P-value Univariate analysis	P-value Log-rank test	P-value Multivariate analysis	Hazard ratio (95% CI)
Substage		0.002*	0.001*	0.905	
a (29)	544 (93-1403)				Ref
b (16)	125 (5-862)				3.375 (1.572-7.247)
Therapy		0.093	0.088	0.002*	
Chemotherapy (21)	211 (5-1403)				1.833 (0.905-3.714)
Chemo-immunotherapy (24)	399 (93-1321)				Ref
PB infiltration		0.015*	0.008*	0.175	
<30% (41)	349 (5-1403)				Ref
≥30% (4)	125 (7-215)				4.017 (1.314-12.283)
LDH activity		0.050*	0.044*	<0.001*	
Normal (24)	560 (111-1403)				Ref

Increased (17)	211 (7-730)				2.172 (1.002-4.710)
BM infiltration		0.035*	0.022*	<0.001*	
<1% (9)	1403 (45-1403)				Ref
1%-20% (33)	337 (5-1321)				2.754 (0.948-7.997)
≥20% (3)	188 (7-215)				7.771 (1.621-37.244)

*=significant result

Fig. 1. Kaplan-Meier curves representing time to progression of 45 dogs with nodal Marginal Zone Lymphoma with <30% (continuous line) or ≥30% (dotted line) peripheral blood flow cytometric infiltration.

Fig. 2. Kaplan-Meier curves representing lymphoma specific survival of 45 dogs with nodal Marginal Zone Lymphoma with <1% (continuous line), 1-20% (dashed line) or ≥20% (dotted line) bone marrow flow cytometric infiltration.