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Original article

Prognostic role of clinical presentation, cytological picture and response to treatment in canine centroblastic lymphoma

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Abstract

Centroblastic lymphoma (CBL) is the most common type of lymphoma in dogs and it usually responds well to chemotherapy. The aim of the study was to provide useful prognostic factors for dogs with CBL. Data regarding sex, breed, age, signalment, treatment and clinical course of the disease from 52 dogs diagnosed with centroblastic lymphoma (CBL) with cytology and immunocytochemistry were provisionally collected and related to the treatment outcome and survival. More than 80% of dogs were treated with chemotherapy and achieved complete remission in 80% of cases. Among the prognostic factors positively related to the overall survival time of dogs with CBL were: the application of chemotherapy, achieving a complete remission, application of at least one additional chemotherapeutic agent to the basic protocol, especially the administration of mitoxantrone and asparaginase. Moreover, mitotic count 14 or higher measured in cytological slides in the area of 2,37 mm² have been linked to shorter overall survival in dogs with CBL.

Key words: lymphoma, prognostic factors, chemotherapy, asparaginase, mitoxantron

Introduction

Lymphoma is the most common neoplasm in dogs which is treatable with chemotherapy (Valli 2013). Dogs with centroblastic lymphoma (CBL) tend to respond well to chemotherapy achieving complete

remission in approximately 80% of cases (Marconato et al. 2015, Childress i wsp. 2018, Davies et al. 2018). Median overall survival time (OS) varies between 8 and 11 months (Childress et al. 2018, Davies et al. 2018, Sierra Matiz et al. 2018), reaching even 17 months in a single study (Ponce et al. 2004).

Data regarding prognostic factors for dogs with CBL are sparse and most of so far carried out studies have applied to a larger group of diffuse B-cell lymphoma (DLBCL). Martini et al. (2015) showed that thrombocytopenia, leukocytosis and lymphocytosis were more frequent in dogs with large B cell lymphoma with massive bone marrow involvement, which in turn has been linked to worse prognosis (Marconato et al. 2013). Anemia, neutrophilia and hyperglobulinemia had negative prognostic value in dogs with DLBCL (Childress et al. 2018, Davies et al. 2018). Higher Lymphocyte-to-Monocyte Ratio (LMR) and lower Neutrophil-to-Lymphocyte Ratio (NLR) were associated with longer OS in dogs with DLBCL treated with chemotherapy (Davies et al. 2018) and lower LMR was linked to shorter time to progression and OS (Marconato et al. 2015). Dogs with DLBCL that were treated only with glucocorticosteroids did not appear to survive longer than untreated dogs whereas the administration of doxorubicin significantly extended OS (Valli et al. 2013). Intensity of neoplastic cell proliferation, expressed as mitotic count or formerly mitotic index, is considered to be a prognostic factor in various neoplasms (for example, in mammary gland carcinomas, soft tissue sarcomas, melanocytic tumors), however, their prognostic value remains unclear in canine lymphomas (Valli et al. 2013, Sierra-Matiz et al. 2018).

The aim of this study was to provide prognostic factors for dogs with CBL easy to obtain during clinical examination, blood tests, cytological examination and clinical course of the disease.

Materials and Methods

The study included dogs presented to two large veterinary clinics in Warsaw in 2009-2016. In this time period, data regarding sex, breed, age, signalment, treatment and clinical course of the disease in dogs with cytologically diagnosed centroblastic lymphoma (CBL) were provisionally collected. Samples for cytological examination were obtained by the fine-needle aspiration or fine-needle non-aspiration biopsy of enlarged lymph nodes, and in cases where blood involvement was suspected on the basis of complete blood count (lymphocytosis and the presence of atypical cells) blood smears from peripheral blood were also prepared. In case of systemic lymphadenomegaly at least three samples were collected from at least two enlarged lymph nodes. Finally, all the dogs with definitive diagnosis of CBL established by two pathologists, and confirmed with immunochemistry were enrolled in the study.

Routine cytological examination was performed on at least 3 smears of each aspirate. They were dried, fixed in 70% methanol, stained with Giemsa solution

and examined using the light microscopy. Smears for the immunocytochemical examination were dried, fixed in acetone at 4°C for 5-10 minutes, and stained immediately or stored at -20°C until testing. Immunocytochemical staining was performed according to Caniatti et al. (1996) and Sapieryński (2010) using commercially available antibodies (Dako® Denmark) for the pan-T-lymphocyte marker CD3 (Polyclonal Rabbit Anti-Human) and B-cell antigen receptor complex CD79 α (Monoclonal Mouse Anti-Human). Two smears from the same case were stained using both antibodies. The intensity of the CD antigen expression in cytological preparations was assessed in the light microscope and the result was considered positive if at least 80% of lymphomatous cells showed a strong cytoplasmic reaction. Negative controls were processed in the same way, using buffer solution instead of primary antibodies. The positive controls for CD3 and CD79 α were cellular samples collected from impression smears of canine hyperplastic lymph nodes.

All cases were immunophenotyped and those expressing CD79 α receptor (immunophenotype B) were classified according to the updated Kiel classification adapted for canine lymphoma as previously described independently by two groups of pathologists (Fournel-Fleury et al. 1997, Fournel-Fleury et al. 2002, Ponce et al. 2004). The following features were determined: size and shape of cells, cytoplasm volume and intensity of cytoplasm staining, size and shape of nuclei, the position of nucleus in a cell; size, distinctness, number and positioning of nucleoli and appearance of nuclear chromatin. Among samples with B-cell immunophenotype those which consisted of blastic lymphocytes with round nuclei with several prominent nucleoli located in the margin and with scant, basophilic cytoplasm were classified as CBL. All cases of CBL were classified as pleomorphic (centroblastic pleomorphic, CBP) due to the admixture of large blasts with round nuclei with one, central nucleoli (immunoblasts) and macronucleolated medium cells (MMC, both types of cells constituted no more than 20%; Fournel-Fleury et al. 1997, Ponce et al. 2004, Sözmen et al. 2005, Valli et al. 2013). Centroblastic pleomorphic were divided according to the predominant cell size into the CBPS (CBP Small Cells) and CBPLC (CBP Large Cells). Microscopic examination was performed with the use of the light microscope Olympus BX41 with ocular field number (FN) 22.

Mitotic count (MC; the number of mitotic figures in 10 high power field, HPF, at the magnification of 40x, in the area of 2.37 mm²) was performed in the fields with the most intense proliferation features.

Prognosis for dogs with CBL was expressed as the OS (time from cytological diagnosis CBL through death).

Table 1. Chemotherapeutic protocols used in 42 dogs.

Chemotherapeutic protocols	n (%) of dogs with CBL
COP	3 (7)
COP + mitoxantrone	1 (2)
COP + mitoxantrone and L-asparaginase	1 (2)
COP + mitoxantrone and L-asparaginase and lomustine	1 (2)
COP + doxorubicin/epirubicin (CHOP)	18 (43)
CHOP + L-asparaginase	2 (5)
CHOP + lomustine	3 (7)
CHOP + mitoxantrone and L-asparaginase	3 (7)
CHOP + mitoxantrone and lomustine	2 (5)
CHOP + L-asparaginase and lomustine	3 (7)
CHOP + mitoxantrone and L-asparaginase and lomustine	5 (12)

Response to chemotherapy was classed as complete remission (size of the lymph nodes decreased by over 50%), partial remission (size of the lymph nodes decreased by 25-50% of the initial size) or no remission.

Characteristics of dogs and treatment protocols

Centroblastic lymphoma was cytologically diagnosed in 52 dogs, which were enrolled in the study – 34 females (65%) and 18 males (35%) at the age of 1-15 years (median 7.5 years, IQR 10-14 years). Age did not differ between males and females ($p=0.486$) nor between purebred and mixed breed dogs ($p=0.812$).

Forty two (81%) dogs were treated with chemotherapy, 7 (13%) were treated only with glucocorticosteroids (GCS) and 3 (6%) were left without any treatment based on the owner's decision.

Glucocorticosteroids treatment included prednisone administered at the daily dose of 0.5 to 2 mg/kg or methylprednisolone administered at the dose of 2 to 5 mg/kg i.m. at 1 week intervals. The therapy was discontinued and the dog was euthanized when its welfare could no longer be maintained with this palliative treatment.

Chemotherapy was administered according to the COP protocol consisting of cyclophosphamide, vincristine, and prednisolone or CHOP protocol with additional doxo- or epirubicin. Pure COP protocol was used in only 3 dogs (7%) and pure CHOP protocol in 18 dogs (43%). In the remaining 21 dogs (50%) at least one additional chemotherapeutic agent was administered. These were L-asparaginase in 15 dogs (36% of 42 dogs), lomustine in 14 dogs (33%) and mitoxantrone in 13 dogs (31%). Of 21 dogs treated with COP/CHOP protocol with an additional drug, 6 dogs received one additional chemotherapeutic agent, 9 dogs – two additional

chemotherapeutic agents, and 6 dogs – three additional chemotherapeutic agents (Table 1). Glucocorticosteroids were administered before the time of cytological diagnosis of CBL in 25 of 42 dogs treated then with chemotherapy (60%).

Statistical analysis

Numerical variables were presented as the median, interquartile range (IQR) and range, and compared between the groups with the Mann-Whitney U test. Categorical variables were presented as a count and percentage of the group, and compared between the groups using the Pearson's chi-square test or Fisher exact test. Overall survival times (OS) were analyzed using plots of the Kaplan-Meier estimator and compared between the groups with the Cox-Mantel test. The relationship between numerical variables and OS was analyzed using the univariable Cox proportional hazard model. A significance level was set at 0.05. The statistical analysis was performed in TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, CA, USA).

Results

Overall survival time

Overall survival was analyzed for 52 dogs with CBL. Three groups of factors possibly affecting OS were analyzed: 1) clinical and laboratory alterations; 2) cytological features of CBL; 3) treatment.

Clinical and laboratory alterations

There was no significant link between clinical presentation or routine blood check-up results and OS.

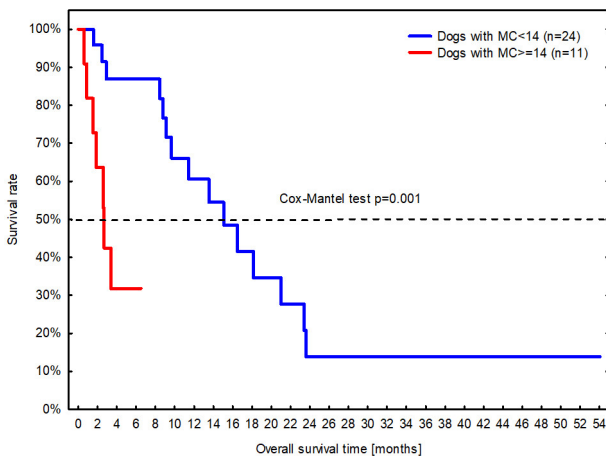


Fig. 1. Kaplan-Meier survival curves of dogs with centroblastic lymphoma (CBL) depending on their mitotic count (MC) on the day of diagnosis

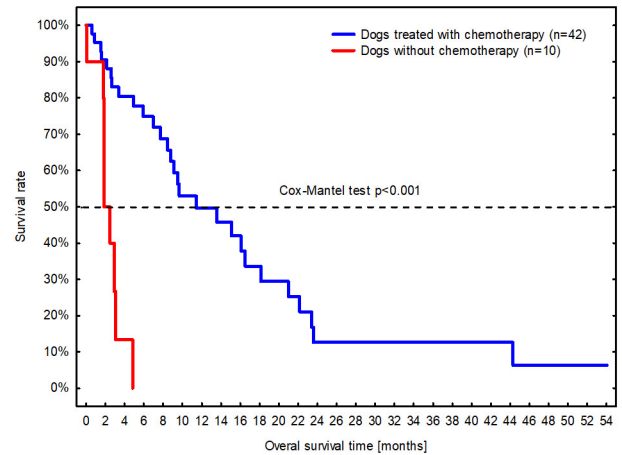


Fig. 2. Kaplan-Meier survival curves of dogs with CBL depending on the type of treatment

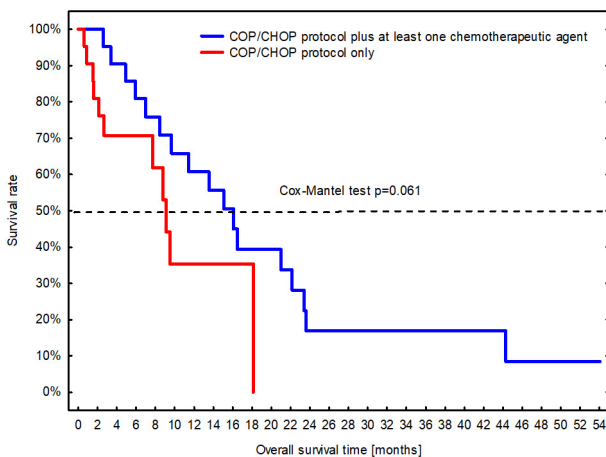


Fig. 3. Kaplan-Meier survival curves of dogs with CBL treated with chemotherapy based on COP/CHOP protocol with (n=21) and without (n=21) additional drug.

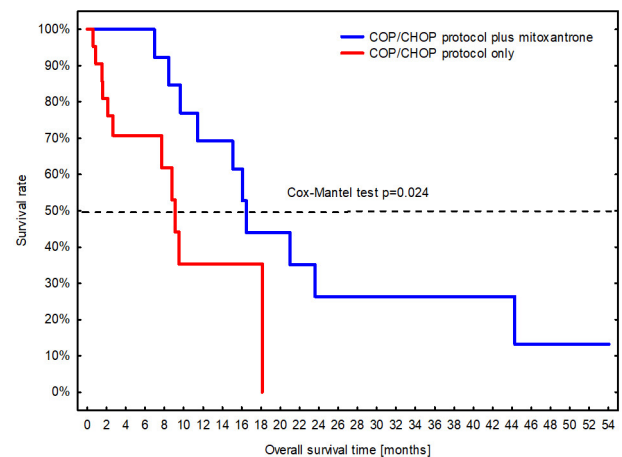


Fig. 4. Kaplan-Meier survival curves of dogs with CBL treated with chemotherapy based on basic COP/CHOP protocol (n=21) and COP/CHOP protocol with mitoxantrone (n=13).

Cytological features of CBL

Of 52 dogs with CBL, 33 (63%) had CBPSC subtype and 19 (37%) had CBPLC subtype. Similar proportion of dogs with each of subtype received chemotherapy – 28 dogs with CBPSC (85%) and 14 dogs with CBPLC subtype (74%) ($p=0.325$). Overall survival did not differ significantly between dogs with different subtypes – median (IQR) OS was 8 (3-15) months in dogs with CBPSC and 12 (3-21) months in dogs with CBPLC ($p=0.212$).

Mitotic count was determined for 35 dogs. Median MC (IQR) was 9 (5-14) and did not differ significantly between dogs with or without chemotherapy ($p=0.676$). Mitotic count was significantly negatively linked to OS (HR=1.14, CI 95%: 1.04, 1.24, $p=0.002$). Dogs with $MC < 14$ lived significantly longer [median OS (IQR) of 15 (9-21) months] than dogs with $MC \geq 14$ [median OS of 2.6 (1.4 to unidentifiable) months; $p=0.001$; Fig. 1].

Treatment

Chemotherapy was significantly linked to the longer OS ($p < 0.001$) – 42 dogs treated with chemotherapy had median (IQR) OS of 11 (6 to 21) months, while median OS of 10 dogs without chemotherapy was only 2 months (Fig. 2).

Basic chemotherapeutic protocols (COP/CHOP) resulted in shorter OS compared to protocols with the use of at least one additional chemotherapeutic agent – median (IQR) OS of 9 (2-12) months vs. 15 (7-23) months, respectively, and this difference was close to statistical significance ($p=0.061$; Fig. 3).

There was no significant difference in OS between chemotherapeutic protocols with one, two or three additional chemotherapeutic agents ($p=0.989$).

Compared to dogs treated with basic chemotherapeutic protocols (CO/CHOP), OS was significantly longer for dogs treated with mitoxantrone (n=13) whose median OS (IQR) was 16 (10-26) months ($p=0.024$;

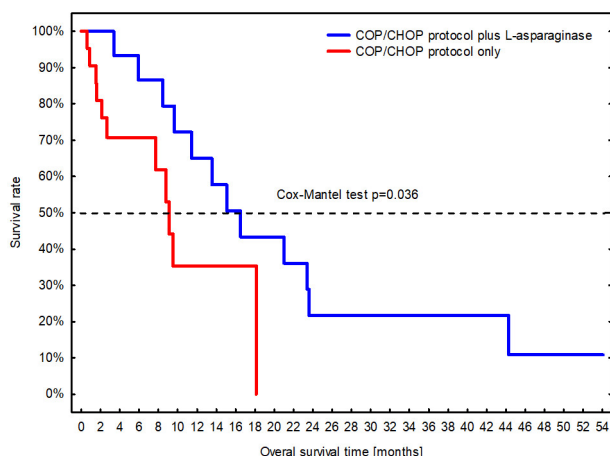


Fig. 5. Kaplan-Meier survival curves of dogs with CBL treated with chemotherapy based on basic COP/CHOP protocol (n=21) and COP/CHOP protocol with L-asparaginase (n=15).

Fig. 4) and dogs treated with L-asparaginase (n=15), whose median OS (IQR) was 15 (9-24) months ($p=0.036$; Fig. 5) but not for dogs treated with lomustine (n=14), whose median OS (IQR) was 9 (5-16) months ($p=0.444$).

Administration of GCS before the diagnosis did not have any influence on the OS ($p=0.267$).

Chemotherapy outcome

Of 42 dogs treated with chemotherapy 34 (81%) achieved complete remission, 5 (12%) – partial remission, and 3 (7%) had no remission. Complete remission was significantly linked to longer OS ($p=0.002$) – median (IQR) OS of dogs with complete remission was 15 months (9 to 22 months), whereas in dogs with partial or no remission median (IQR) OS was only 3 months (1 to 6 months; Fig. 6).

Three groups of factors possibly affecting chemotherapy outcome were analyzed: 1) clinical and laboratory alterations; 2) cytological features of CBL; 3) treatment.

Clinical and laboratory alterations

Dogs with complete remission had significantly higher lymphocyte count (median of 1.4, IQR 1.0 to 2.1) compared to dogs with partial or without remission (median of 0.9, IQR: 0.7 to 1.1; $p=0.036$). No other differences in the clinical examination or routine blood check-up results were found.

Cytological features of CBL

Neither cytological subtype nor MC were significantly linked to complete remission ($p=0.232$ and $p=0.688$, respectively).

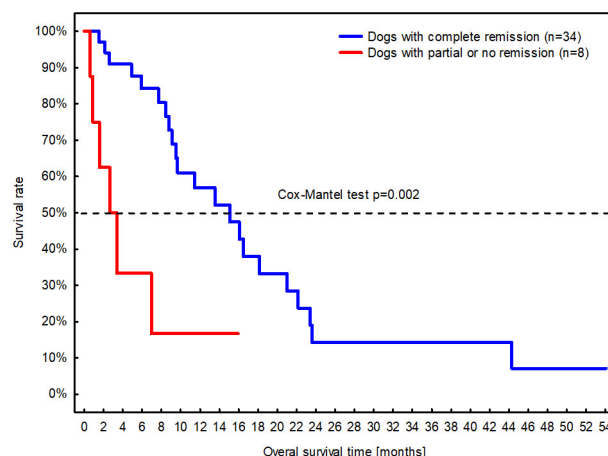


Fig. 6. Kaplan-Meier survival curves of dogs undergoing chemotherapy for CBL depending on their remission status.

Chemotherapeutic protocol

Sixteen of 21 dogs treated with basic COP/CHOP protocol had complete remission (76%). The complete remission was observed in 14 of 15 dogs treated with additional L-asparaginase (93%), 12 of 14 dogs treated with additional lomustine (86%), and 11 of 13 dogs treated with additional mitoxantrone (86%). None of those proportions was significantly higher than observed when basic chemotherapeutic protocols were used ($p=0.367$, $p=0.676$, and $p=0.682$, respectively). Moreover, complete remission was observed in 19 of 25 dogs (76%) which received early GCS treatment and in 15 of 17 dogs (88%) which did not and this difference was not significantly different ($p=0.439$).

Discussion

In the article we reported valuable prognostic factors for dogs with CBL that can be easily defined during routine diagnostic procedures. Prognostic significance was found for the value of MC which can be easily counted during microscopic evaluation of cytological slides.

Chemotherapy in dogs with CBL is usually based on CHOP protocol with possible additional administration of L- asparaginase, lomustine, or mitoxantrone. The vast majority of dogs in our study were treated with CHOP-based protocol. COP was administered mainly in dogs in which the risk of cardiotoxicity of doxorubicin was considered as unacceptably high.

Median OS of dogs treated with chemotherapy was almost a year, and increased to 15 months in dogs with complete remission. These figures are consistent with results of previous studies (Childress et al. 2018, Davies et al. 2018), however, less optimistic than those previ-

ously reported by Ponce et al. (2004) where median OST in dogs with complete remission exceeded one and half of a year. These differences can be to some extent explained by the fact that in the work of Ponce et al. (2004) most of the dogs presented lower clinical stage of lymphoma than those in our study (IIIb vs. IVb).

No prognostic significance of cytological subtype (CBPSC or CBPLC) was found, and therefore it seems to be of no practical value to divide CBL according to predominant cell size. On the other hand, mitotic count proved a significant prognostic factor. Dogs with MC of 14 or more all lived shorter than 7 months, compared to dogs with MC of less than 14 whose OS varied between very broad limits. In the research of Valli et al. (2013) shorter OST was found in dogs with MC 21 or more in histopathological slides. Unfortunately, calculations included dogs with all morphological types of lymphoma for which the value of MC was available. Moreover, the prognostic significance of this value was not proven in the later research including 29 dogs with DLBCL (Sierra-Matiz et al. 2018). It must be noted, that the area of microscopic fields in which MC was counted was not given in any of these papers. Great emphasis is currently placed on the standardization of microscopic field to the area of 2.37 mm², as the results differ significantly depending on optical parameters of ocular (Meuten i wsp. 2016).

Expectedly, the administration of chemotherapy significantly prolonged OS. Our study confirms that complete (not partial) remission extends OS of dogs with CBL (Davies et al. 2018, Childress et al. 2018). Unfortunately, the numbers of dogs treated with various combinations of chemotherapeutic agents were too small to clearly indicate if there was any particularly effective drug.

Data concerning the impact of drugs administered additionally to basic chemotherapeutic protocol on the prognosis in dogs with centroblastic or even B-cell lymphoma are scarce. In one study (Childress et al. 2018) more than a half of the dogs received L-asparaginase but no influence on survival was found. In other studies data were usually collected from dogs with "multicentric lymphoma" without any morphotype given, sometimes even without immunophenotyping of neoplastic cells. In one research there was an impact of administration of L-asparaginase on achievement of complete remission in dogs with multicentric lymphoma (Jeffreys et al. 2005). Unfortunately, connection between administration of L-asparaginase and OS was not investigated. In the research of Brodsky et al. (2009) addition of L-asparaginase to MOPP protocol (mechlometamine, vincristin, prednisone, procarbazine) significantly prolonged OS of dogs with T-cell lymphoma with hypercalcemia. In our study administration

of L-asparaginase significantly prolonged OS of dogs with CBL. Substituting doxorubicin with mitoxantrone allowed to reduce side effects without affecting OS in the research of Wang et al. (2016). In our study mitoxantrone administered additionally to basic protocols significantly prolonged OS of dogs with CBL.

The response to the treatment was similar to that reported recently by Davies et al. (2018) and Childress et al. (2018).

In conclusion, clinical presentation or routine blood check-up results do not have prognostic significance in dogs with CBL. Administration of glucocorticosteroids before administration of chemotherapy does not affect prognosis. The MC in cytological result helps to distinct dogs with significantly worse prognosis. Chemotherapy extends life of dogs with CBL and the addition of mitoxantrone and L-asparaginase to the basic protocol is recommended. Consideration of further treatment should be given in dogs that have not achieved complete remission after chemotherapy course due to the poor prognosis.

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