Paraneoplastic Syndrome as a Prognostic Factor in Dogs with Multicentric Lymphoma

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ABSTRACT

To evaluate the correlation between paraneoplastic syndrome and clinical outcomes, 54 dogs with multicentric lymphoma that received the same induction chemotherapy, including cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisolone, were retrospectively analyzed. Paraneoplastic syndrome was defined as a diagnosis of anemia, neutrophilic leukocytosis, thrombocytopenia, or hypercalcemia at the first blood examination and cancer cachexia and fever at first presentation in dogs with lymphoma. The complete remission rate and progression-free interval were significantly higher and longer in dogs without paraneoplastic syndrome than in dogs with paraneoplastic syndrome (P = .046 and P = .024), respectively. Anemia was significantly associated with a decreased progression-free interval in univariable Cox regression analysis and decreased survival time in multivariable Cox regression analyses (P = .036 and P = .041). Multivariable Cox regression analyses revealed that dogs with thrombocytopenia had significantly longer survival time (P = .016). These findings indicate that anemia can be a negative prognostic factor and thrombocytopenia can be a positive prognostic factor in canine lymphoma.

INTRODUCTION

Lymphoma is one of the most common neoplasms in dogs, accounting for 7%-24% and 83% of all canine neoplasms and hematopoi-

etic tumors, respectively.[1, 2] The incidence of canine lymphoma is rising, probably because of improved diagnostic methods, aging of the dog population, and exposure to some risk factors.[3, 4] Many chemotherapy protocols have been designed and used in dogs with lymphoma. The protocols based on C-cyclophosphamide, H-hydroxydaunorubicin, O-vincristine, and P-prednisolone (CHOP) have been widely used by most veterinarians. Canine lymphoma is highly chemoresponsive and the remission rate is greater than 85%. The reported survival time ranges from 8 to 12 months.[5, 6]

Paraneoplastic syndrome (PNS) represents neoplasm-associated alterations that produce and release biologically active substances to influence body structure or function or both; these effects occur distant from the primary tumor.[7, 8] Several PNSs have been reported in the veterinary literature. The most common PNS in companion animals include:

- hypercalcemia
- hypoglycemia
- anemia
- erythrocytosis
- thrombocytopenia
- leukocytosis
- cachexia
- alopecia
- myasthenia gravis
- fever
- hypertrophic osteopathy, and
- gastrointestinal ulceration.[7]

PNS must be recognized because some PNSs are related to oncologic emergencies and must be treated before or concurrently with the treatment of the primary tumor. Several PNSs disappear after appropriate treatment, and they reappear with tumor relapse.[9]

Several prognostic factors have been studied in dogs with lymphoma. The reported prognostic factors include lymphocyte phenotype, tumor stage, substage, hypercalcemia, anatomic location, histopathology grade, mitotic index, proliferating cell nuclear antigen, aneuploidy and proliferation indices.[10-12] Some of these prognostic factors are unavailable in some countries that without laboratory support. This study evaluated the importance of PNS as a potential prognostic factor in dogs with multicentric lymphoma.

MATERIALS AND METHODS

Study Population Selection and Evaluation

This retrospective study included dogs with cytological or histopathological diagnoses of large- to intermediate-cell multicentric lymphoma at the National Taiwan University Veterinary Hospital from May 2010 to June 2018. Dogs with other concurrent diseases and those receiving corticosteroid or chemotherapy before diagnosis were excluded. The breed, sex and neuter status, age, body weight, body temperature, stage, substage, immunophenotype, complete blood cell count, serum albumin, and calcium concentration were recorded at the first time of chemotherapy. Radiography and abdominal ultrasound were performed to investigate other lymphoid tissue, liver and spleen involvement. The immunophenotype was determined by flow cytometry or immunohistochemistry. Clinical staging was performed according to the World Health Organization (WHO) staging criteria for canine lymphoma 13. Bone marrow aspiration was not routinely performed unless the CBC indicated bone marrow involvement.

PNS was defined as a diagnosis of anemia, neutrophilic leukocytosis, thrombocytopenia, or hypercalcemia at the first blood examination and cancer cachexia or fever at first presentation in dogs with lymphoma. The diagnosis of anemia (hematocrit < 37.3%), neutrophilic leukocytosis (white blood cell > 16.76 103/μl and neutrophil > 11.64 103/μl), thrombocytopenia (platelet < 200 103/μl), and hypercalcemia (total calcium > 12 mg/dL or ionized calcium > 1.5 mmol/L) were based on the results of blood examination and compared to the reference in the laboratory of our hospital. The diagnosis of cachexia was made if the patient lost

Table 1. *Characteristics of dogs with and without PNS.*

	PNS (n=37)	Non-PNS (n=17)	*P*-value
Age (years)	8.3	6.9	0.077
Body weight (kg)	21.9	15.4	0.089
Sex			0.144
Female	15 (40.54%)	11 (64.71%)	
Male	22 (59.46%)	6 (35.29%)	
Clinical stage			
V	6 (16.22%)	0 (0.00%)	0.161
IV	17 (45.95%)	7 (41.18%)	0.777
III	14 (37.84%)	9 (52.94%)	0.379
II	0 (0.00%)	1 (5.88%)	0.315
Substage			
b	11 (29.73%)	1 (5.88%)	0.078
T cell	1 (3.70%, 1/27)	0 (0.00%, 0/13)	1

Abbreviations: PNS, paraneoplastic syndrome.

weight (> 10%) prior to presentation and with a blood test result of hypoalbuminemia (albumin < 2.3 g/dL) when presentation. The fever was diagnosed as body temperature over 39.5 °C when presentation.

Treatment and response assessment

All dogs were treated with a multi agent CHOP chemotherapy protocol. Chemotherapy was delayed for 1 week if neutropenia (< 3000 cells/μL) or thrombocytopenia (< 100000 cells/μL) was identified in the pretreatment assessment. If the chemotherapy continuation was contraindicated in the animal due to the clinical condition, the treatment was postponed, and a reevaluation was conducted after 1 week.

Treatment response was determined based on lymph node measurement. A complete response (CR) was characterized by the complete disappearance of all measurable disease; a partial response (PR) was characterized by a >30% decrease but a < 100% decrease in the mean sum longest diameter of target lesions; a stable disease (SD) was characterized by a <30% decrease or <20% increase in target lesions; and progressive disease (PD) was characterized by a >20% increase in target lesions or the development of a new lesion [14].

Progression-free interval (PFI) was calculated from the initiation of chemotherapy to the time of disease progression. Survival time (ST) was calculated from the initiation of chemotherapy to the time of patient death. The data of dogs were censored from the analysis of the remission time if they were alive during remission, death before relapse, or were lost to follow-up during remission. The data of dogs were censored from the survival analysis if they died from causes other than lymphoma, loss to follow-up, or if were alive at the end of the study.

Statistical analysis

Age and body weight were compared using Student's t test. Sex, proportion of dogs in each group with known prognostic factors (stage, substage, and immunophenotype), and response rate were compared using Fisher's exact test. The median PFI and median ST were determined using Kaplan-Meier analysis; the differences between the PNS group and no-PNS group were assessed using the log-rank test, and the estimated survival curves were generated using the product-limit method. Cox proportional hazard regression was used to estimate

Table 2. *Response and outcome rates in PNS and no-PNS groups.*

	PNS (n=37)	No-PNS (n=17)	*P*-value
Response rate			
Overall (CR + PR)	33 (89.2%)	17 (100.0%)	0.296
CR	29 (78.4%)	17 (100.0%)	0.046
PR	4 (10.8%)	0 (0%)	0.296
SD	4 (10.8%)	0 (0%)	0.3
Median progression free survival	203 days	299 days	0.024
Median survival time	262 days	506 days	0.117

Abbreviations: CR, complete response; PD, progressive disease; PNS, paraneoplastic syndrome; PR, partial remission; SD, stable disease.

Table 3. *Univariable cox regression analysis of paraneoplastic syndromes associated with progression free survival and survival time in dogs with multicentric lymphoma.*

	Progression Free Survival		Survival Time	
Parameters	HR (95% CI)	*P*	HR (95% CI)	*P*
Anemia	1.87 (1.04-3.37)	0.036	1.58 (0.83-3.01)	0.168
Neutrophilic leukocytosis	1.43 (0.76-2.67)	0.267	1.66 (0.80-3.44)	0.170
Thrombocytopenia	1.30 (0.69-2.44)	0.413	0.67 (0.31-1.42)	0.294
Cachexia	1.73 (0.75-4.01)	0.198	1.86 (0.70-4.94)	0.215
Fever	1.02 (0.46-2.30)	0.954	1.10 (0.48-2.52)	0.820

Abbreviations: CI, confidence interval; HR, hazard ratio.

the effect of PNS on PFI and ST by using univariable models first and then multivariable models. All statistical analyses were considered significant at $P < .05$.

RESULTS

This study included 54 dogs diagnosed with large- to intermediate-cell multicentric lymphoma. Fifty dogs were diagnosed by cytology and 4 dogs were diagnosed by histopathology. Most dogs were mixed breed (n = 17), followed by Golden retriever (n = 11), Beagle (n = 5), Welsh Corgi (n = 4), Schnauzer (n = 4), Dachshund (n = 2), Maltese (n = 2), Shih Tzu (n = 2), and one each of Border Collie, Cocker spaniel, Siberian Husky, Toy Poodle, Rottweiler, West Highland White Terrier, and Yorkshire Terrier.

A total of 37 dogs had been diagnosed with at least one PNS. The patients' signalment, clinical stage, substage, and immunophenotype are listed in Table 1. The age (mean ± SD) of the dogs with PNS and without PNS was 8.3 ± 2.8 years and 6.9 ± 2.4 years, respectively (P = .077). The body weight (mean ± SD) of the dogs in the PNS and no-PNS groups was 21.9 ± 13.5 kg and 15.4 ± 10.4 kg (P = .089), respectively. There were 15 female (5 intact) and 22 male (10 intact) dogs with PNS and 11 female (4 intact) and 6 male (3 intact) dogs without PNS. There were no significant differences in sex (P = .144), clinical stage (P = .161; P = .777; P = .379 and P = .315), and substage (P = .078). In the PNS group, only 27 dogs received immunophenotyping, and only one dog was diagnosed with T cell lymphoma. In the no-PNS group, none of the 13 dogs who underwent immunophenotyping was diagnosed with T cell lymphoma (P = 1).

Anemia, neutrophilic leukocytosis, thrombocytopenia, and hypercalcemia were diagnosed in 25, 17, 15, and 0 dogs with lymphoma. Eight dogs were diagnosed with cancer cachexia and another 8 dogs with

Table 4. *Multivariable cox regression analysis of paraneoplastic syndromes associated with progression free survival and survival time in dogs with multicentric lymphoma.*

	Progression Free Survival		Survival Time	
Parameters	HR (95% CI)	*P*	HR (95% CI)	*P*
Anemia	2.00 (0.97-4.09)	0.059	2.27 (1.03-5.00)	0.041
Neutrophilic leukocytosis	1.17 (0.58-2.33)	0.662	1.64 (0.74-3.63)	0.221
Thrombocytopenia	0.82 (0.36-1.89)	0.646	0.31 (0.12-0.80)	0.016
Cachexia	1.39 (0.56-3.46)	0.484	2.02 (0.66-6.12)	0.216
Fever	0.81 (0.32-2.07)	0.664	1.07 (0.44-2.62)	0.884

Abbreviations: CI, confidence interval; HR, hazard ratio.

fever at the first presentation.

The treatment response and clinical outcome are listed in Table 2. In PNS group, 29 dogs (78.4%) exhibited CR, 4 dogs (10.8%) exhibited PR, and 4 dogs (10.8%) exhibited SD. In the no-PNS group, 17 dogs (100%) exhibited CR. The overall response rate (CR + PR) was 89.2% in the PNS group and 100% in the no-PNS group (P = .296). CR was significantly higher in the no-PNS group than in the PNS group (P = .046). The median PFI and ST in PNS group were 203 and 262 days, and those in no-PNS group were 299 and 506 days (P = .024 and P = .117), respectively.

In univariable Cox regression analysis, only anemia was significantly associated with decreased PFI (P = .036), whereas other univariable models for PFI or ST revealed no significance (Table 3). The results of multivariable Cox regression analyses for the association of PNS with PFI and ST are listed in Table 4. The results revealed that anemia was significantly associated with decreased ST (P = .041), and dogs with thrombocytopenia had significantly longer ST (P = .016).

DISCUSSION

PNS that have been commonly reported in canine lymphoma include hypercalcemia, anemia, thrombocytopenia, and cancerous cachexia [12, 15, 16]. We added another 2 PNSs, neutrophilic leukocytosis and fever, in this study to evaluate the influence of PNS on the treatment response. Dogs with lymphoma and without PNS had a significantly higher complete remission rate and longer PFI than those with lymphoma and PNS. No significant difference was observed in other known negative prognostic factors between the PNS group and the no-PNS group. Therefore, PNS can be a negative prognostic factor for the complete remission rate and progression time in canine multicentric lymphoma.

To determine the importance of each PNS in canine lymphoma, we used univariable and multivariable Cox regression for further analysis. Anemia is a common complication in human patients with various tumor types and can be used as a negative factor for treatment response and prognosis [17]. The same conclusion has been reported in veterinary medicine, which revealed that anemia is a risk factor for poor response and prognosis in dogs with lymphoma [18]. However, the induction chemotherapy protocols were different in the previous study. Variable protocols may influence the final result. Therefore, we used the same protocol to treat dogs with lymphoma in our study. Our result revealed that anemia was related to decreased remission time and ST in dogs with lymphoma that underwent the same chemotherapy.

Thrombocytopenia is the other common PNS in canine lymphoma. The mechanism includes platelet consumption, increased platelet destruction, or decreased platelet production [7]. However, spontaneous hemorrhage was not common in dogs with lymphoma. Multivariable Cox regression

analysis revealed that dogs with lymphoma and thrombocytopenia had significantly longer ST. The exact relationship was not fully understood. The same conclusion was stated in other veterinary studies that showed thrombocytopenia was significantly associated with an increased ST in dogs with multicentric lymphoma that received CHOP chemotherapy [6, 19]. Thrombocytopenia has previously been identified as a positive prognostic factor for OST in dogs with NHL that received CHOP chemotherapy. However, this result was conflicted with another study, which revealed thrombocytopenia was associated with poor prognosis in dogs with lymphoma [16]. Some human studies have demonstrated that platelets play several roles in all steps of tumorigenesis, including tumor growth, tumor cell extravasation, and metastasis. Patients with cancer and thrombocytosis are associated with shorter survival time [20, 21]. The number of dogs with thrombocytopenia in our study (n = 15) and in childress study (n = 22) were small, therefore, further research is needed to classify the different causes of thrombocytopenia in dogs with lymphoma and to confirm and explain this finding by comparing the clinical outcomes.

Paraneoplastic neutrophilic leukocytosis in dogs with lymphoma was caused by colony-stimulating factors [7]. If we rule out causes that can induce neutrophilic leukocytosis, such as infection and inflammation, we suspect that the neutrophilic leukocytosis was induced by the tumor. At first evaluation, chest radiography, abdominal ultrasound and urine culture was performed and there were no evidence of infection and inflammation in a total of 8 dogs with neutrophilic leukocytosis. Six dogs with neutrophilic leukocytosis were also diagnosed as fever. However, this PNS did not significantly influence the clinical outcome in our study.

Cancer cachexia is caused by metabolic alterations even with proper nutrition support. Changes in protein metabolism can result in the increased turnover and decreased synthesis of new protein. Alterations in lipid metabolism include increased lipolysis and decreased activity of lipoprotein lipase. Alteration in carbohydrate metabolism results in increased lactate and insulin resistance. These alterations lead to energy deprivation[9]. Patients often show weight loss and muscle atrophy for a period. There were only 8 dogs (14.8%) diagnosed as cachexia in our study, which was lower than human research that cachexia diagnosed in 40% to 90% hospitalized patient [22]. However, we did not find any significant difference in remission time and ST in dogs with cancer cachexia.

The mechanism of fever in patients with cancer is related to the release of pyrogenic cytokines by either the tumor or as a result of the host immune response to the tumor[7]. Body temperature usually returns to normal after appropriate treatment is provided. In our study, the presence of fever was not related to prognosis in dogs with lymphoma.

This study has some limitations. The sample size was small, making it possible to ignore some potential significance. Furthermore, this was a retrospective study, and immunophenotype was not examined in all dogs. Immunophenotype is a valuable prognostic factor in remission and survival for dogs with lymphoma. Lower response rate and shorter remission and ST were correlate with T-cell immunophenotype [12]. There were only one dog of T-cell lymphoma in our study. The possible explanation is that B-cell phenotype is dominate in canine multicentric lymphoma; only 17% to 28% of lymphoma were diagnosed as T-cell origin [23]. The other reason is that T-cell lymphoma have a worse outcome than B-cell lymphoma. Some owner decided euthanasia rather than treating the dogs. However, these shortage may influence the study results.

In conclusion, PNS can be used as a prognostic factor in dogs with multicentric lymphoma. Lymphoma dogs with anemia had a worse outcome than dogs without

anemia. However, thrombocytopenia was a positive prognostic factor in canine lymphoma

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Conflict of interest

The authors confirm that they do not have any conflict of interest

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