NOTE *Internal Medicine*

Hyperferritinemia is associated with short survival time in dogs with multicentric lymphoma

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ABSTRACT. In the present study, we examined the relationship between serum ferritin concentration before treatment and survival time in dogs with multicentric lymphoma. Eighteen dogs with multicentric lymphoma were enrolled in the study. When the dogs were classified into high and low ferritin groups on the basis of their serum ferritin concentration (3,000 *n*g/m*l* cut-off value), the median survival time of dogs with high concentrations (≥3,000 *n*g/m*l*, n=7) was 40 days, whereas it was 360 days among dogs with low concentrations (<3,000 *n*g/m*l*, n=11). This difference was statistically significant (P=0.001). This finding suggests that the initial high level of serum ferritin indicates short survival time in dogs with multicentric lymphoma. Large-scale research is necessary to confirm this finding.

KEY WORDS: canine, ferritin, lymphoma, prognostic factor

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Canine lymphoma is relatively common, accounting for 7–24% of canine malignancies and 83% of canine hematopoietic tumors [17]. Numerous prognostic factors for canine lymphoma, including World Health Organization (WHO) clinical stage, immunophenotype, histopathological malignancy, anatomical location, pretreatment history (including corticosteroids) and presence of anemia (packed-cell volume [PCV] <35%), have been evaluated [11, 17]. Identification of these prognostic factors is important for prognosis prediction as well as for treatment planning.

Ferritin is an iron storage protein that is ubiquitous in all mammals. It has an iron core within a 24-mer globular protein complex consisting of heavy (H chain) and light (L chain) subunits with molecular masses of 21 and 19 kDa, respectively [7, 19]. Ferritin can accommodate 3,000–4,500 iron molecules and protects cells from reactive oxygen species [8]. In mammals, including dogs, ferritin normally circulates in the serum at a relatively low concentration (<1 μg/m*l*), and the serum ferritin concentration is positively correlated with the level of iron stored in the body [9, 15]. In veterinary medicine, it has been reported that canine serum ferritin levels also increase in histiocytic sarcoma, splenic hemangiosarcoma, immune-mediated hemolytic anemia and lymphoma [4, 5, 10, 14].

In humans, serum ferritin concentration has been reported as an important prognostic factor associated with a short survival time in patients with non-Hodgkin's lymphoma [20]. Serological markers potentially indicative of the prognosis of canine lymphoma, including C-reactive protein, glutathion-S-transferase, thymidine kinase and vascular endothelial growth factor [17], have been studied previously, but to date, no study has evaluated the relationship between serum ferritin concentration and survival time in canine lymphoma cases.

Data for this study were obtained from medical records of 34 dogs with multicentric lymphoma referred to the Kitasato University Veterinary Teaching Hospital Small Animal Medical Center between July 2008 and March 2013. A diagnosis of multicentric lymphoma was confirmed by cytological or histopathological examination, and the multicentric form of the disease was confirmed by the swelling of more than one body surface lymph node. All dogs were performed physical examination, complete blood count, serum biochemistry, urinalysis, thoracic and abdominal radiography, abdominal ultrasonography and lymph node aspiration with or without biopsy. Bone marrow aspiration or biopsy was not performed, and tumor involvement to liver and/or spleen was evaluated by ultrasonographic imaging. Immunophenotyping of each lymphoma was carried out by a clonality assay using polymerase chain reaction (PCR), which was performed by a commercial laboratory (North Labo, Sapporo, Japan). None of the dogs underwent immunohistochemical analysis. Survival time of the dogs was determined from diagnosis by a review of their medical records. We excluded dogs that were previously treated with chemotherapeutic drugs, including corticosteroids, for lymphoma at their local clinics or were not followed-up. All dog owners provided their written informed consent for including their dogs in this study, which was approved by the Kitasato University Small Animal Committee.

All dogs were treated with a modified version of the UW-Madison protocol for canine lymphoma without using L-asparaginase [6]. At first relapse of the disease, the previous protocol was repeated. At second relapse, the rescue

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protocol was performed, and the dogs were administered lomustine and L-asparaginase as first choice, and dexamethasone, melphalan, actinomycin D and cytosine arabinoside as second choice [2, 16]. Response for the first chemotherapy induction was determined according to WHO criteria [12]. In brief, complete remission (CR) was defined as complete resolution of measurable tumors; partial remission (PR) as a ≥50% reduction in tumor size with no new lesions; stable disease (SD) as a 25–50% reduction in tumor size; and progressive disease (PD) as a ≥25% increase in tumor size or the development of new lesions.

A total of 5 m*l* of blood was collected from the cephalic or jugular vein of each dog at the initial visit to our hospital. Two m*l* was anti-coagulated with heparin and centrifuged at 1,640 × *g* for 5 min to separate the plasma for immediate plasma alanine transaminase (ALT) activity testing with an auto analyzer (AU400; Beckman Coulter, Brea, CA, U.S.A.); 1 m*l* was mixed with ethylenediaminetetraacetic acid to determine the PCV using an automatic analyzer (Celltac αMEK-6358; Nihon Kohden, Tokyo, Japan); and 2 m*l* was left at room temperature for 30 min and then centrifuged at 1,560 × *g* for 5 min to separate the serum. The sera and plasma samples were stored at −20°C until use. Serum ferritin concentration was measured using sandwich enzyme-linked immunosorbent assay (ELISA) using purified canine heart ferritin as a standard and purified rabbit anti-canine heart ferritin polyclonal antibody, as described in our previous report [3].

The data were analyzed using XLSTAT-Pro (Version 2013, Addinsoft, New York, NY, U.S.A.). Pearson's correlation test was used to analyze correlations between the serum ferritin concentration and plasma ALT activity or PCV. Kaplan–Meier curves of survival time were generated and compared between the groups separated on the basis of serum ferritin concentration using the log-rank test. Multivariate analysis of the prognosis was performed using Cox's proportional-hazards regression model. Results were considered significant when the *P* value was <0.05.

Eighteen dogs were enrolled in the present study. The cause of death of all dogs was associated lymphoma, and none was euthanized. Eight were male (6 intact, 2 castrated), and 10 were female (5 intact, 5 neutered). The breeds were as follows: Mongrel (n=3), Golden Retriever (n=3), Miniature Schnauzer (n=2), Papillon (n=1), Bulldog (n=1), Flat-coated Retriever (n=1), Beagle (n=1), Toy Poodle (n=1), Miniature Dachshund (n=1), Welsh Corgi (n=1), Bernese Mountain Dog (n=1), French Bulldog (n=1) and Chihuahua (n=1). The body weight of the dogs ranged from 2.1 to 52 kg (median, 11.2 kg), and they ranged in age from 3 to 13 years (median, 7.5 years). Twelve dogs had B-cell lymphoma (positive monoclonality of gamma globulin heavy chain gene expression), and 6 dogs had T-cell lymphoma (positive monoclonality of T-cell receptor gene expression). Fourteen of these dogs were ultrasonographically confirmed to have abnormality in the liver and/or spleen, suggesting that most dogs were presented at an advanced stage (WHO clinical stage >IV), and 4 of these dogs (cases 10, 11, 15 and 16) had to undergo rescue protocol. The median serum ferritin concentration in all 18 dogs with multicentric lymphoma before treatment was 1,932 *n*g/m*l* (range, 924–14,154 *n*g/m*l*). Data for each dog are shown in Table 1.

The 18 dogs with multicentric lymphoma were divided into groups according to the serum ferritin concentrations (<3,000 *n*g/m*l* and ≥3,000 *n*g/m*l*). The overall survival rate was calculated at each 500 *n*g/m*l* interval starting at a serum ferritin concentration of 1,500 *n*g/m*l*. The overall survival

Table 1. Clinical data of dogs with multicentric lymphoma

Case No.	Breed	Body weight (kg)	Age (y)	Gender	Serum ferritin (*n*g/m*l*)	PCV (%)	ALT (U/*l*)	Immuno-phenotype	Tumor involvement to liver and/or spleen	Response	Survival time (d)
1	Golden Retriever	31.3	4	Female	14,154	31.5	29	B	Yes	PD	11
2	Papillon	3.5	4	Male*	13,579	21.3	109	B	Yes	CR	67
3	Miniature Schnauzer	4.8	13	Female	7,702	34.3	36	B	Yes	PD	9
4	Bulldog	34.1	4	Male*	7,597	35.8	221	T	Yes	PR	34
5	Flat-coated Retriever	26	9	Female	4,049	31.9	21	T	No	PR	61
6	Beagle	12.5	4	Male*	3,408	34	30	B	Yes	PR	80
7	Mongrel	11	9	Female*	3,122	28.2	55	B	Yes	NR	40
8	Golden Retriever	30	10	Male*	2,454	41	114	T	Yes	PR	105
9	Golden Retriever	33.2	7	Female*	2,320	54.8	133	T	No	CR	730 (Alive)
10	Toy Poodle	5.6	3	Male	1,544	41.6	38	B	Yes	CR	511
11	Miniature Dachshund	5.6	10	Female*	1,390	56.7	12	B	Yes	CR	415
12	Welsh Corgi	11.3	13	Male*	1,322	43.8	60	B	Yes	NR	17
13	Bernese Mountain Dog	52	5	Male	1,239	40.9	235	B	No	NR	28
14	Mongrel	15	7	Male*	1,199	32.3	35	T	Yes	PR	112
15	French Bulldog	9.9	5	Female*	1,115	46.2	174	B	Yes	CR	670
16	Mongrel	10.9	8	Female*	982	45.9	24	B	Yes	CR	360
17	Chihuahua	2.1	11	Female	962	40.2	61	T	No	PR	215
18	Miniature Schnauzer	5.7	12	Female	924	46.5	57	B	Yes	CR	540 (Alive)

*: Intact.

Table 2. Prognistic factors in univariate and multivariate analysis in dogs with multicentric lymphoma. Survival analysis was performed according to the Kaplan-Meier method and log-rank test. Multivariate analysis was performed using Cox's proportional-hazards regression model

Characteristic	No. of patients	Median survival time (days)	Univariate analysis	Multivariate analysis	
			P value	hazard ratio	*P* value
Age (y)					
<7	7	160	0.502	0.25	0.26
≥7	11	112			
Sex					
Male	8	74	0.134	4.61	0.47
Female	10	288			
Body Weight (kg)					
<10	7	415	0.182	1.46	0.58
≥10	11	61			
PCV (%)					
<35	7	61	0.009	1.54	0.68
≥35	11	360			
Immunophenotype					
T	6	109	0.719	0.43	0.43
B	12	74			
Serum ferritin (*n*g/m*l*)					
<3,000	11	360	0.001	16.49	0.045
≥3,000	7	40			
Tumor involvement to spleen and/or liver					
Yes	14	93	0.647	0.78	0.83
No	4	138			

rates were significantly different when the serum ferritin concentration was 3,000 *n*g/m*l* ($P<0.01$). Consequently, we considered 3,000 *n*g/m*l* as the cut-off value for serum ferritin levels in the present study. In univariate analysis, overall survival time was significantly short for dogs that had PCV <35% ($P=0.009$) and serum ferritin level ≥3,000 *n*g/m*l* ($P=0.001$, Table 2). When the dogs were classified by serum ferritin cut-off value (3,000 *n*g/m*l*), Kaplan–Meier survival curves showed that the median survival time for the dogs in the high serum ferritin group (n=7) was 40 days, while that in the low serum ferritin group (n=11) was 360 days (Fig. 1). Multivariate analysis showed that serum ferritin level was the only independent prognostic factor for overall survival time in dogs with multicentric lymphoma in the present study (Table 2).

We evaluated the response to chemotherapy, and response rate (CR or PR) for initial chemotherapy protocol in each high (≥3,000 *n*g/m*l*, n=7) and low serum ferritin group (<3,000 *n*g/m*l*, n=11) was 57% and 82%, respectively. Therefore, the difference in response to therapy in each group did not significantly affect their survival time. All 4 dogs in which rescue protocol was performed belonged to the low serum ferritin group, and none in the high serum ferritin group, because of their poor general condition. In this study, it was thought that most of the dogs with lymphoma were at an advance clinical stage (more than stage IV); therefore, the relationship between the clinical stage and serum ferritin level was not determined.

In human patients with non-Hodgkin's lymphoma, hyperferritinemia at the time of pretreatment suggests a negative prognostic factor [20]. Similar to this finding, we found that serum ferritin concentration >3,000 *n*g/m*l* predicted poor prognosis in dogs with multicentric lymphoma. However, the mechanism of hyperferritinemia in cancers, including lymphoma, and the exact relationship between high serum ferritin concentration and disease prognosis are not well understood. A study reported that extracellular ferritin stimulates iron-independent proliferation of human breast cancer

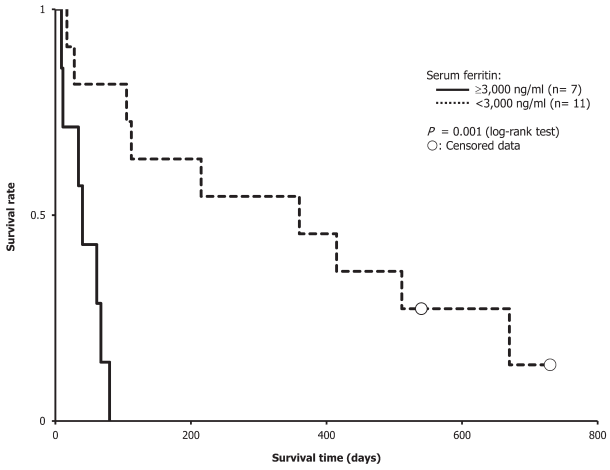

Fig. 1. Kaplan–Meier survival curves of dogs with multicentric lymphoma and high (n=7, ≥3,000 *n*g/m*l*, straight line) or low (n=11,<3,000 *n*g/m*l*, dashed line) serum ferritin concentrations.

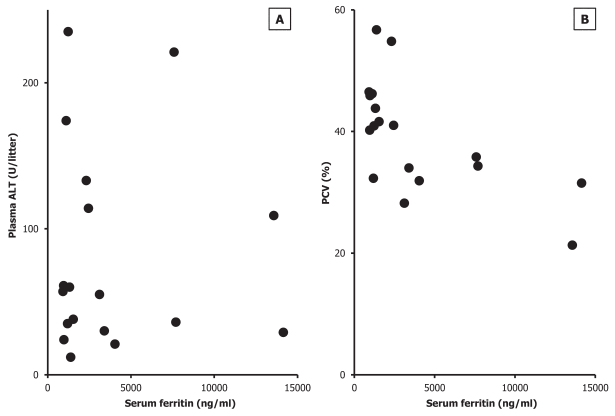

Fig. 2. Correlations between serum ferritin concentration and plasma ALT activity (A; r=0.032, *P*=0.64) or PCV value (B; r= −0.68, *P*=0.005) in dogs with multicentric lymphoma.

cell lines [1], which suggests that hyperferritinemia may influence tumor progression. Interestingly, serum ferritin level was negatively correlated with PCV (r=−0.68, *P*=0.005). In addition, similar to the previous report [11], dogs with PCV <35% showed short overall survival time on univariate analysis in this study. Ferritin synthesis is mainly regulated at the translational level by intracellular iron and at the transcriptional level by inflammatory cytokines, such as tumor necrosis factor [8]. Therefore, ferritin is regarded as one of the acute-phase proteins [19]. Inflammation-related mild hypoproliferative anemia, which is called anemia of chronic disease, was related to an increase in the serum ferritin levels [18]; therefore, high serum ferritin level may be associated with inflammation caused by the lymphoma. However, more detailed studies are needed to prove this hypothesis. On the other hand, the liver diseases result in high serum ferritin levels, because hepatocyte contains high levels of ferritin [5]. However, it was thought that the high serum ferritin concentration was not related to hepatocyte injury (r=0.032, *P*=0.64), because no correlation between serum ferritin and plasma ALT activity, which is as an indicator of hepatocyte injury, was identified [13] (Fig. 2).

The presence of high serum ferritin levels before treatment was suggested to be a predictive factor for poor prognosis in dogs with multicentric lymphoma. This was a small-scale study, and further studies for elucidating the mechanism of hyperferritinemia are required.

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