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Clinical Characteristics and Prognostic Factors in Dogs with Histiocytic Sarcomas in Japan

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ABSTRACT. Canine histiocytic sarcoma (HS) is a rare neoplasm that originates from dendritic cells or macrophages, and there have been a number of cases experienced in Japan. To identify the characteristics and prognostic variables that determine outcome in dogs with HS in Japan, medical records of 73 dogs with HS were retrospectively analyzed. Signalment, clinical signs, complete blood count (CBC), blood chemistry profiles, treatment, response to treatment and overall survival (OS) were analyzed. Diagnosis of HS was determined histologically in 44 cases and cytologically in 29 cases. The most frequently diagnosed breeds were Flat-Coated Retrievers (*n*=16, odds ratio [OR] 62.0), Pembroke Welsh corgis (*n*=15, OR 9.7) and Bernese Mountain dogs (*n*=14, OR 45.0). Median survival time for all dogs in this study was 43 days. In the dogs that received no treatment or only symptomatic treatment, the median OS was 12 days (range 2–254 days) compared with that of dogs that received surgical treatment and/or chemotherapy (85 days, range 4–360 days). Univariate analysis identified anemia, thrombocytopenia, hypoalbuminemia, hypoproteinemia and not receiving antitumor treatment (chemotherapy and/or surgery) as factors significantly associated with shorter OS. Multivariate analysis confirmed that platelet counts, localized/disseminated lesional pattern and whether the dog received antitumor treatment were significantly predictive of survival.

KEY WORDS: canine, histiocytic sarcoma, prognostic factor, retrospective study.

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Reactive and neoplastic histiocytic disorders have been described in dogs. These include reactive histiocytosis, systemic histiocytosis, histiocytoma, localized histiocytic sarcoma (HS) and disseminated HS. Canine HS is a rare round cell neoplasm originating from dendritic cells or macrophages [1, 6], and localized HS and disseminated HS are malignant histiocytic tumors. In addition, hemophagocytic HS was recently described as a different subtype of HS that arises from macrophages and has an aggressive clinical course [6].

Localized HS is reported to occur most commonly in the bone, joints, skin and subcutaneous tissues [13]. The majority of dogs with localized HS eventually develop distant metastases to the spleen, liver, lymph nodes, bone marrow and lung, even if the primary tumor is treated with localized therapies (surgery and/or radiation) [10, 13]. Therefore, chemotherapy is usually administered for both localized and disseminated HS. However, studies evaluating responses to chemotherapy in canine HS are limited. Although there have been several reports on responses to chemotherapy using doxorubicin [14], liposomal doxorubicin [14], paclitaxel [9] and CCNU [10, 12, 13], survival time of dogs with HS has been short so far despite the use of aggressive treatments. In addition, no study has compared the prognosis in canine HS between dogs that received antitumor treatments with surgery and/or chemotherapy and those that did not receive such antitumor treatment. Knowledge of the prognostic factors in canine HS also remains limited to date, although one study has reported that anemia, thrombocytopenia, hypoalbuminemia and splenic involvement were associated with a worse prognosis in dogs with HS [12].

The Bernese Mountain dog, Rottweiler, Dobermann, Golden Retriever, Labrador Retriever and Flat-Coated Retriever exhibit a higher prevalence of HS than other breeds [1, 11]. In addition, it has been shown that systemic histiocytosis and HS are inherited in the Bernese Mountain dog with a hereditary coefficient of 0.298 [8]. One study of subdural HS in Japan reported that Pembroke Welsh corgi was the most frequently diagnosed breed [5]. However, epidemiological studies on the incidence of canine HS in Japan are limited. The objective of the present study was to examine the clinical characteristics, outcome and prognostic factors of canine HS in Japan.

MATERIALS AND METHODS

Animals: Medical records of dogs that were referred to

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Table 1. The dog breeds most commonly diagnosed with HS in this study and the associated odds ratio values

Breed	Number of dogs	Odds ratio	95% CL
Flat-Coated Retriever	16	62.0	37.6–102.3
Pembroke Welsh corgi	15	9.7	5.6–17.0
Bernese Mountain dog	14	45.0	26.3–77.2
Golden Retriever	8	5.0	2.4–10.3
Labrador Retriever	6	3.0	1.3–7.0
Others	14		

CL: Confidence Limits

the Veterinary Medical Center at the University of Tokyo (UT-VMC) from April 2007 to July 2012 and diagnosed with HS by cytology or histopathology were reviewed. All histopathological examinations were performed by one pathologist (K. UCHIDA). Cases were diagnosed with HS based on the morphological features described in a report by Affolter and Moore [1]. Immunohistochemical staining was performed when possible for confirmation of the diagnosis. Antibodies against human leukocyte antigen (HLA)-DR alpha-chain were used in 5 dogs (mouse anti-human monoclonal antibody; Dako Japan, Tokyo, Japan), ionized calcium-binding adaptor molecule 1 (Iba1) in 3 dogs (rabbit anti-human polyclonal; Wako., Osaka, Japan), CD3 in 2 dogs (rabbit anti-human polyclonal., Dako Japan), CD204 in 1 dog (mouse anti-human monoclonal antibody; TransgGenic. Inc., Kumamoto, Japan) and CD20 in 1 dog (rabbit anti-human polyclonal., NeoMarkers, Fremont, CA, U.S.A.). Cytochemical staining for alpha-naphthyl butyrate esterase (α-NBE) and inhibition of the enzyme by sodium fluoride were also performed when possible (in 7 dogs) as a marker of monocyte/macrophage lineage.

Clinical information: Information extracted from medical records included signalment, clinical signs, complete blood cell counts (CBCs), blood chemistry profile, diagnostic methods, treatments, response rates and survival times. Adverse events during chemotherapy were evaluated using the Veterinary Cooperative Oncology Group − Common Terminology Criteria for Adverse Events v1.0 (VCOG-CTCAE) [15]. Dogs with measurable lesions were evaluated for response using Response Evaluation Criteria in Solid Tumors [7]. Responses to chemotherapy were categorized as follows: complete response (CR); complete disappearance of all measurable disease, partial response (PR); >30% but <100% reduction in the sum of the longest diameters of measurable tumors, stable disease (SD); <30% reduction or <20% increase in the sum of the longest diameters of measurable tumors without the appearance of new neoplastic lesions and progressive disease (PD); an increase of >20% in the sum of the longest diameters of measurable tumors or the appearance of new neoplastic lesions [7]. Overall survival (OS) was defined as the duration from the date of diagnosis to the date of death from any cause, and response duration was defined as the duration from the documentation of response (CR or PR) to the date of relapse or progression. OS and response duration for dogs lost to follow-up were censored at the date they were last known to be alive.

Statistical analysis: Survival probabilities were estimated using the Kaplan–Meier product limit method. In the examination of prognostic factors, log-rank tests were used to determine whether each factor as assessed at diagnosis influenced survival. In addition, a forced entry Cox proportional hazards model was developed to assess the independent contributions of various prognostic factors. Several prognostic factors yielding a P value of less than 0.1 were included in the hazards model. A value of $P<0.05$ was considered to be significant in all statistical tests. Data were analyzed using commercially available statistics software (JMP, version 4, The Statistical Discovery Software, SAS Campus Drive, Cary, NC, U.S.A.).

RESULTS

Animals: Medical records of 73 dogs with HS were reviewed in this study. The mean age was 9.6 years (range, 1.9–15.2 years), and the mean body weight was 23.5 kg (range 2.6–55 kg). Twenty-two dogs were intact males, 14 were castrated males, 13 were intact females, and 24 were spayed females. There were 16 Flat-Coated Retriever, 15 Pembroke Welsh corgis, 14 Bernese Mountain dogs, 8 Golden Retrievers, 6 Labrador Retrievers, 3 Shih Tzu, 2 Shetland sheepdogs and 1 each of Beagle, Pointer, French Bulldog, Rottweiler, Maltese, Yorkshire Terrier, Miniature Dachshund, Toy Poodle and mixed breed dogs. The odds ratios (ORs) of those dogs against all dogs admitted to UT-VMC for the same period were 62.0 (95% confidence limits [CL], 37.6–102.3) for Flat-Coated Retrievers, 9.7 (95% CL 5.6–17.0) for Pembroke Welsh corgis, 45.0 (95% CL 26.3–77.2) for Bernese Mountain dogs, 5.0 (95% CL 2.4–10.3) for Golden Retrievers and 3.0 (95% CL 1.3–7.0) for Labrador Retrievers (Table1).

Diagnoses were obtained histologically in 44 cases and cytologically in 29 cases. In cytological examinations, the majority of cells had abundant and lightly basophilic cytoplasm, and some had multiple small cytoplasmic vacuoles. These cells had pleomorphic nuclei with vesicular chromatin and multiple nucleoli. Multinucleated giant cells, atypical mitotic figures and phagocytosis were commonly observed, although the frequencies of those observations varied among the cases. Histopathologically, the features of HS were also characterized by the irregular proliferation of pleomorphic histiocytic cells and multinucleated giant cells combined with various inflammatory reactions. In 7 dogs where cy-

Table 2. Distribution of documented tumor lesions (localized HS and disseminated HS)

Organ affected	Number of dogs	
	Localized HS	Disseminated HS
Spleen	8	11
Lung	6	10
Lymph node (as primary site)	5 (1)	9 (0)
Bone/joint	11	3
Skin and soft tissues	9	4
Liver	1	4
Central nervous system	2	2
Kidney	0	4
Others	3	4

tochemical analysis was performed, the tumor cells were α-NBE-positive, and the positive staining was inhibited by the addition of sodium fluoride, indicating the tumor cells originated from the monocyte/macrophage lineage. In the immunochistochemical staining performed in 6 dogs, the tumor cells were positive for HLA-DR, Iba-1 and/or CD204. Forty-one dogs were diagnosed with localized HS, and 32 were diagnosed with disseminated HS.

Clinical characteristics: Frequent clinical signs at presentation were anorexia (34%, *n*=25), lameness (29%, *n*=21), lethargy (23%, *n*=17), cough (15%, *n*=11), presence of one or more palpable masses (11%, *n*=8), diarrhea (8%, *n*=6), dyspnea (5%, *n*=4) and vomiting (5%, *n*=4). CBC and blood biochemistry profile were examined in 73 dogs. Clinicopathologic abnormalities found at the first presentation included anemia (HCT <30%, 18/73), thrombocytopenia (PLT <100,000/*μl,* 18/73), hypoalbuminemia (Alb <2.6 g/d*l*, 18/49), azotemia (BUN >29.2 mg/d*l*, 18/72), hypercreatininemia (CRE >1.4 mg/d*l*, 5/72), hyperbilirubinemia (T-Bil >0.5 mg/d*l*, 18/19), increased liver enzymes (ALT >78 U/*l,* 30/72), increased C-reactive protein (CRP>1.0 mg/d*l,* 54/67) and increased fibrin/fibrinogen degradation products (FDP) (FDP >5.0 *μ*g/m*l*, 7/33). Radiographic and ultrasonographic examinations were performed in 68 and 44 dogs, respectively, before treatment. In addition, computed-tomography (CT) and magnetic resonance imaging (MRI) were performed in 26 and 5 dogs, respectively.

The distribution of the tumor lesions examined in this study is summarized in Table 2. Nineteen of the 73 (26%) dogs had lesions in the spleen, 16 (22%) in the lung, 14 (19%) in lymph nodes, 14 (19%) in bones and/or joints, 13 (18%) in skin/soft tissue, 5 (7%) in the liver, 4 (5%) in the central nervous system, 4 (5%) in the kidney, 3 (4%) in the mediastinum, 3 (4%) in the gastrointestinal tract and 1 (1%) in the oral cavity. In addition, frequently affected organs were examined in each of the breeds including the Flat-Coated Retriever, Pembroke Welsh corgi and Bernese Mountain dog. Of 16 Flat-Coated Retriever, 9 (56%) had localized HS and 7 (44%) had disseminated HS, and the frequently affected organs in this breed were the skin/soft tissue (7 dogs, 44%), lymph nodes (6 dogs, 38%) and lung (5 dogs, 31%). Of 15 Pembroke Welsh corgis, 13 (86%) had localized HS and 2 (14%) had disseminated HS, and the frequently affected organs in this breed were the lung (8 dogs, 53%) and spleen (3 dogs, 20%). Of 14 Bernese Mountain dogs, 9 (64%) had localized HS and 5 (36%) had disseminated HS, and the frequently affected organs in this breed were the spleen (6 dogs, 43%) and bone/joint (4 dogs, 29%). Of the 73 dogs in this study, 11 were considered to have a hemophagocytic subtype based on hematologic and clinicopathologic abnormalities. However, it was difficult to diagnose definitively, because immunohistochemical staining (MHC class II and the leuko-integrin CD11d/CD18, etc.) using fresh or frozen samples would have been required for a confirmed diagnosis of this subtype.

Treatments: Treatment information was available for 69 of the 73 dogs in the present study. Twenty-two (30%) received no treatment or only symptomatic treatment after diagnosis, and 1 dog was euthanized before treatment. Surgical treatment was performed in 16 dogs (21%), of which 10 received adjuvant chemotherapy. Thirty (41%) received only chemotherapy. Chemotherapy protocols using CCNU (lomustine) were performed as a single agent (23 dogs) or in combination with ACNU (nimustine; 2 dogs) or doxorubicin (1 dog). ACNU was used as a single agent (12 dogs) or in combination with L-asparaginase (2 dogs). The mean dosage of CCNU was 63.7 mg/m^2(range, 23.2–93.0 mg/m^2), and the median number of administrations was 2.5 (range, 1–10). Among the 26 dogs that received CCNU, 9 experienced neutropenia (grade 1, 2 dogs; grade 2, 1 dog; grade 3, 2 dogs; grade 4, 4 dogs), 2 experienced thrombocytopenia (grade 1, 1 dog; grade 4, 1 dog), 2 experienced vomiting (grade 1, 2 dogs), 8 showed elevation of liver enzyme activity (alanine aminotransferase, ALT) (grade 2, 1 dog; grade 3, 5 dogs; grade 4, 2 dogs), and one exhibited grade 1 diarrhea, as adverse events after CCNU treatment. The mean dosage of ACNU was 30 mg/m^2(range 25–40 mg/m^2), and the median number of administrations was 1.5 (range, 1–8). Of the 16 dogs that received ACNU, 3 experienced neutropenia (grade 1, 1 dog; grade 4, 2 dogs), one exhibited grade 2 vomiting, and 2 showed elevation of ALT activity (grade 1, 1 dog; grade 3, 1 dog), as adverse events after ACNU administration. There was no significant relationship between the kind of chemotherapeutic agent administered and the occurrence of adverse events.

Among the dogs that received treatment with chemotherapy alone, response to treatment could be objectively measured in 17 dogs. In the 11 dogs that received CCNU as a single agent, the response rate was 55% (6/11) (CR, 1; PR, 5), and the median response duration was 111 days (range, 35–291 days). In the 6 dogs that received ACNU as a single agent, the response rate was 50% (3/6) (CR, 1; PR, 2), and the median response duration was 48 days (range, 29–99 days). There was no response recorded after treatment with L-asparaginase or doxorubicin.

Outcomes: Of the 73 dogs analyzed in the present study, 12 were censored, because of loss to follow up. The median OS for all dogs in the study was 43 days (range, 2–468 days). In the dogs that had no treatment or only symptomatic

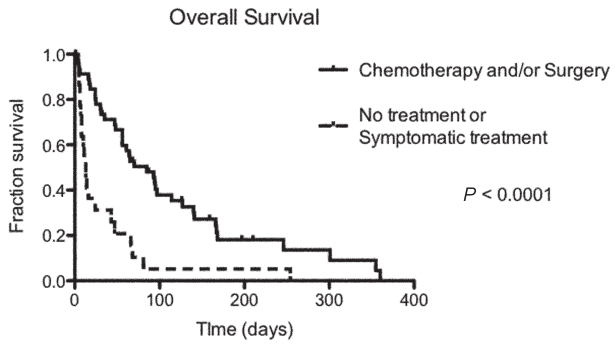

Fig. 1. Kaplan–Meier curve showing the difference in survival between the dogs that received antitumor treatments (chemotherapy and/or surgery) and the dogs that received only symptomatic treatment or no treatment.

treatment, the median OS was 12 days (range, 2–254 days) compared with that of dogs who received surgical treatment and/or chemotherapy (85 days, range, 4–360 days) (Fig. 1). For the dogs that underwent surgery only (n=6), chemotherapy only (n=30), and both surgery and chemotherapy (n=10), median OS was 91, 76 and 62.5 days, respectively. Univariate analysis identified anemia, thrombocytopenia, hypoalbuminemia, hypoproteinemia and not receiving antitumor treatment (chemotherapy and/or surgery) as factors significantly associated with shorter survival times (Table 3). Median OS of anemic dogs (PCV <30%) was 24 days compared with 61 days in dogs without anemia (P=0.0097), and median OS of dogs with thrombocytopenia (platelets <100,000 /μl) was 10 days, compared with 66 days without thrombocytopenia (P=0.0005). Median OS of dogs with hypoalbuminemia (Alb <2.6 g/dl) was 18 days compared with 64 days in dogs without hypoalbuminemia (P=0.0302), and median OS of dogs with hypoproteinemia (TP <5.0 g/dl) was 18 days compared with 64 days in dogs without hypoproteinemia (P=0.0007). The significant prognostic factors were then determined from combinations of the 5 factors; thrombocytopenia, disseminated distribution of lesion, no antitumor treatment, hypoalbuminemia and anemia, using Cox's proportional hazards modeling. The results in this study suggest that a combination of thrombocytopenia (OR, 5.7), no antitumor treatment (OR, 3.5) and existence of disseminated lesion (OR, 2.0) may be the most appropriate for prognostication (Table 4). There were 11 dogs that were conceivably affected with hemophagocytic HS (OS 18 days, range 1–64 days), and their prognoses tended to be poorer than those of the other dogs, even though 6 of the 11 received chemotherapy or surgery.

DISCUSSION

In the present study, clinical characteristics of dogs with HS in Japan were investigated. The signalments of the dogs included were generally similar to those reported in previous studies of canine HS [10, 12, 13]. However, the present study revealed that the Pembroke Welsh corgi was a breed at comparatively high risk of HS in Japan. A previous study including 15 cases of subdural HS in Japan [5] reported that the breed most frequently diagnosed with subdural HS was the Pembroke Welsh corgi (7 of the 15 dogs). It may be necessary to recognize the Pembroke Welsh corgi as a breed at comparatively high risk of developing HS, not only subdural but also other types of HS, in Japan. Although the mode of inheritance of canine HS is not well understood and the genes likely to be involved are unknown, it has been proposed that systemic histiocytosis and disseminated HS (previously called malignant histiocytosis) have familial aspects, and it has been suggested that these diseases may have a genetic basis in the Bernese Mountain dog [8]. Genetic predisposition may also be involved in Pembroke Welsh corgi in Japan.

The distribution of HS in the present study most commonly included the spleen, lung, lymph node, bone/joint skin and/or soft tissues. These results are similar to those reported by Skorupuski *et al.* [12]. Some studies have reported that distribution of the lesions tended to be different between the breed and generally localized in Flat-Coated Retrievers and Golden Retrievers, which was in contrast to Bernese Mountain dogs and Rottweilers, in which HS was invariably disseminated [1, 3, 4]. In the present study, Pembroke Welsh corgis were generally affected with localized HS, while Bernese Mountain dogs and Flat-Coated Retriever did not exhibit a clear tendency with regard to the distribution of lesions.

CCNU was reported to be effective as a chemotherapeutic agent for dogs with HS, and it is the only drug with proven efficacy against HS [12, 13]. One retrospective study reported that 46% of dogs with HS that were treated with CCNU responded to the agent, and the median remission duration was 85 days. In the present study, a similar response rate (55%) was obtained in dogs that received CCNU as a single agent. In addition, clinical responses were obtained in 50% of dogs treated with ACNU as a single agent in the present study. There is currently no literature on the clinical use of ACNU and associated adverse events in veterinary medicine. In the present study, among severe adverse events, grade 4 hematological toxicity was confirmed in only 2 of the dogs (13%) that received ACNU administration, compared to 5 of the dogs (19%) that received CCNU. Hepatotoxicity shown as elevation of ALT was observed after administration of ACNU and CCNU; however, its frequency tended to be lower after ACNU administration. In contrast to CCNU, which is currently available as capsules for oral administration, ACNU can be intravenously injected. Thus, ACNU is easy to administer in cases with vomiting or with gastrointestinal lesions, and doses can be reduced for dogs that experience adverse events. Because of the retrospective nature of the present study, doses and intervals between treatments with ACNU were variable, and adverse events were not fully evaluated; however, it may be worthwhile evaluating dose limiting toxicity, maximum tolerated dose and the efficacy of ACNU administration in the treatment of canine HS.

Of the prognostic factors, hypoalbuminemia, anemia, thrombocytopenia and not receiving surgery and/or chemotherapy were significantly associated with a poor prognosis.

Table 3. Association of variables with survival after a logrank test (significance, $P<0.05$)

Variable	Risk factor (upside)	Number of dogs	Median survival days	*P* value
Age (n=73)	>8 years old	20	56	0.5783
	<8 years old	53	47	
Sex (n=73)	Male	36	56	0.7307
	Female	37	47	
Hematocrit (n=73)	HCT <30%	17	24	0.0097
	HCT ≥30%	56	61	
WBC (n=73)	WBC >17,000 /μl	32	18	0.0576
	WBC ≤17,000 /μl	41	70	
PLT (n=72)	PLT <100,000 /μl	18	10	0.0005
	PLT ≥100,000 /μl	54	66	
TP (n=72)	TP >5.0 g/d*l*	10	18	0.0007
	TP ≤5.0 g/d*l*	62	64	
Alb (n=49)	Alb <2.6 g/d*l*	18	18	0.0302
	Alb ≥2.6 g/d*l*	31	64	
BUN (n=72)	BUN >29.2 mg/d*l*	18	16	0.5744
	BUN ≤29.2 mg/d*l*	54	61	
CRE (n=72)	CRE >1.4 mg/d*l*	5	24	0.5773
	CRE ≤1.4 mg/d*l*	67	56	
ALT (n=72)	ALT >78 mg/d*l*	29	46	0.7284
	ALT ≤78 mg/d*l*	43	56	
CRP (n=66)	CRP >1.0 mg/d*l*	53	33	0.2164
	CRP ≤1.0 mg/d*l*	13	61	
FDP (n=33)	FDP >5 μg/m*l*	7	43	0.4917
	FDP ≤5 μg/m*l*	26	33	
Distribution (n=73)	Localized HS	41	56	0.0692
	Disseminated HS	32	43	
Treatment (n=69)	No treatment or symptomatic treatment	23	11	<0.0001
	Chemotherapy and/or surgery	46	85	

WBC: White Blood Cell, PLT: Platelet count, TP: Total plasma protein, Alb: Albumin, BUN: Blood urea nitrogen, CRE: Creatinine, ALT: Alanine aminotransferase, CRP: C-reactive proteinm, FDP: Fibrin/fibrinogen degradation products.

Table 4. Variables included in the model of survival produced by multivariate Cox proportional hazard analysis

	HR[a)]	95% CL[b)]	*P* value
Thrombocytopenia	5.7	2.3–14.8	0.0002
Disseminated HS	2.0	1.0–4.0	0.0456
Hypoalbuminemia	1.4	0.7–2.9	0.3151
Anemia	0.8	0.4–1.9	0.6729
No antitumor treatment	3.5	1.6–7.4	0.0015

a) HR; Hazzard Ratio. b) CL; Confidence limits.

These results are similar to those of a previous report on canine HS that investigated treatment with CCNU [12], with the exception of not receiving surgery and/or chemotherapy. Hypoalbuminemia, anemia and thrombocytopenia as negative prognostic factors might be associated with hemophagocytic subtype. In a previous study, hemophagocytic HS arising from macrophages was reportedly marked by an aggressive clinical course dominated by splenomegaly, regenerative anemia, thrombocytopenia, hypoalbuminemia and hypocholesterolemia [6]. It was reported that hypoalbuminemia was significantly more severe in the dogs with hemophagocytic HS than in those with nonhemophagocytic HS [6]. Another possibility reported was that hypoalbuminemia might indicate severe inflammation due to tumor invasion. Thrombocytopenia may also result from reduced production due to tumor invasion into bone marrow, increased platelet consumption due to hypercoagulability and immunologic platelet destruction. However, the association of these abnormalities with thrombocytopenia could not be examined in the present study, because the number of dogs

where bone marrow aspiration and blood coagulation tests (prothrombin time, activated partial thromboplastin time and FDP) were performed at first presentation was small. Only 4 of 33 dogs that had coagulation tests were diagnosed as suspected-DIC (Disseminated Intravascular Coagulation) based on diagnostic criteria described by Carr *et al*. [2], and bone marrow aspiration was performed in 1 dog. Multivariate analysis confirmed that platelet counts, localized/disseminated lesional pattern and receiving antitumor treatment were independent prognostic factors. The results obtained in the present study suggested that complete staging is needed before treatment, to predict prognosis. In addition, it was also indicated that prolonged survival could be expected in dogs with HS that were treated with surgery and/or chemotherapy as compared with only symptomatic treatment or no treatment. However, receiving antitumor treatment was a prognostic factor that was independent of other prognostic factors, and there may be an inherent bias in the selection of treatment based on the degree of clinical symptoms. Short survival time (11 days) in the dogs with no treatment or only symptomatic treatment in this study, as reported in a previous study on Flat-Coated Retrievers [4], confirmed that canine HS is rapidly progressive with a grave prognosis. However, the small numbers of subjects enrolled in each treatment (chemotherapy alone, surgery alone, chemotherapy and surgery) made it impossible to confirm which was the best treatment for dogs with HS.

One limitation of the present study was the lack of immunohistochemical staining using anti-CD18 and CD11d antibodies for confirmation of the diagnosis of HS. However, in cases where it was difficult to diagnose HS cytologically or histopathologically, cytochemical analysis for alpha-naphthyl butyrate esterase staining and inhibition of this enzyme by sodium fluoride or immunohistochemistry were assessed to acquire support for a diagnosis of HS, and all were deemed consistent with HS.

In conclusion, HS is an aggressive disease, and the survival times of dogs with HS might be very short if only symptomatic treatment or no treatment is performed. In Japan, it is necessary to recognize that the Pembroke Welsh corgi is a breed at comparatively high risk of HS. Anemia, hypoalbuminemia and thrombocytopenia are negative prognostic indicators as has been previously reported [12]. In addition, the present study revealed that receiving treatment including surgery and/or chemotherapy (CCUN or ACNU) improved the prognosis of dogs with HS as compared with dogs that received only symptomatic treatment or no treatment.

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