NOTE *Internal Medicine*

Hyperferritinemia in Dogs with Splenic Hemangiosarcoma

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ABSTRACT. Serum ferritin concentration increases in dogs in association with various diseases. In this study, we measured serum ferritin levels in dogs with splenic masses, using a sandwich ELISA assay. Eleven dogs with hemangiosarcoma (HSA), six with hematoma, 1 with hemangioma and 3 with lymphoma were enrolled. All dogs with HSA had serum ferritin concentrations above the normal limit (1,357 *n*g/m*l*, mean + 2× standard deviation of normal). Increased serum ferritin concentrations have also been observed in few cases of hematoma, hemangioma and lymphoma. Therefore, hyperferritinemia is not specific for splenic HSA, but may have clinical usefulness as a sensitive test for the disease. Further evaluation of serum ferritin concentrations in dogs with splenic HSA is needed.

KEY WORDS: hemangiosarcoma, serum ferritin, tumor marker.

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Hemangiosarcoma (HSA) is a common malignant tumor in dogs that arises from vascular endothelial cells. HSA is the most common splenic mass in dogs, accounting for 51–66% of all splenic neoplasms, and is highly metastatic. The 12-month survival rate of dogs treated with splenectomy and chemotherapy is below 10%, and the disease has a poor prognosis [17]. Radiographic or ultrasound examinations have commonly been used to detect splenic HSA as a splenic mass. However, these examinations are not generally performed for the case that a clinical symptom is not recognized. Therefore, it is hoped that a useful method of serologic testing in veterinary medicine is carried out. In a prior study, we used sandwich ELISA [7] testing to measure serum ferritin concentration in a large sample of clinical cases, and we found that splenic HSA frequently caused hyperferritinemia.

Ferritin, a ubiquitous iron storage protein, has an iron core within a 24-mer globular protein consisting of heavy (H) and light (L) subunits with molecular masses of 21 kDa and 19 kDa, respectively [9, 20]. Ferritin can accommodate 3,000–4,500 iron atoms and protects cells from reactive oxygen species [10]. All mammalian cells contain ferritin with high concentrations found in the liver, spleen and bone marrow [11]. In normal mammals, including dogs, ferritin circulates in the serum at a relatively low concentration (<1 μg/m*l*), and serum ferritin concentration is positively correlated with body iron storage [1, 3, 19]. Serum ferritin levels change in diseases, such as iron overload, inflammatory diseases, liver damage and malignancies [20]. In veterinary medicine, it has been reported that serum ferritin levels increase in dogs with lymphoma, histiocytic sarcoma and immune-mediated hemolytic anemia (IMHA) [8, 12, 15]. However, there are no reports on serum ferritin concentrations in dogs with splenic HSA. Our purpose in this study was to examine the potential clinical usefulness of serum ferritin measurement as a tumor marker in dogs with splenic masses.

We studied 21 dogs with splenic masses that visited Kitasato University Veterinary Teaching Hospital Small Animal Medical Center between 2008 and 2012. The splenic masses were observed by radiographic or ultrasound examination, and the dogs were treated with splenectomy. Tumors were diagnosed by histopathological examination of resected tissue. All dog owners provided written informed consent for participation in this study, which was approved by the Kitasato University Small Animal Committee. Two m*l* of blood were collected from the cephalic or jugular vein of each dog before surgery and held at room temperature for 30 min to coagulate before centrifugation for 5 min at 1,640 × *g* to separate serum. The obtained serum sample was preserved at −20°C until serum ferritin testing. The other blood sample was anti-coagulated with heparin and centrifuged again for 5 min at 1,640 × *g* to separate plasma for immediate plasma alanine transaminase (ALT) activity testing using an auto analyzer (AU400; Beckman Coulter, Brea, CA, U.S.A.). Serum ferritin concentration was measured by sandwich enzyme-linked immunosorbent assay (ELISA) using the method described in our previous report [7]. All results are given as mean ± standard deviation (SD). Pearson's correlation test was performed to analyze the correlation between serum ferritin concentration and plasma ALT activity.

The individual data of the dogs with splenic masses are shown in Table 1. The dogs were classified as clinical stage 1, 2 or 3, based on the results of the presurgical medical examination, using the staging guidelines described in a previous report [16]. Stage 1 was a tumor confined to the primary site, stage 2 was a tumor with rupture or regional lymph node metastasis and stage 3 included any tumor with gross distant metastasis or multicentric disease. Eleven dogs with

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Table 1. Clinical data in dogs with splenic masses

Case No.	Breed	Age (years)	Sex	Clinical stage	Diagnosis	Hematcrit (%)	ALT (U/*l*)	Serum ferritin (ng/m*l*)
1	Mongrel	11	Spayed female	2	Hemangiosarcoma	27.6	62	4,549
2	Labrador retriever	13	Spayed female	1	Hemangiosarcoma	26.6	167	3,149
3	Yorkshire terrier	12	Female	2	Hemangiosarcoma	25.9	40	6,514
4	Golden retriever	9	Castrated male	2	Hemangiosarcoma	22.7	42	9,146
5	Golden retriever	9	Castrated male	2	Hemangiosarcoma	20.0	31	7,840
6	Labrador retriever	8	Female	3	Hemangiosarcoma	22.4	29	4,285
7	Golden retriever	9	Female	1	Hemangiosarcoma	45.3	149	4,667
8	Shetland sheepdog	11	Spayed female	3	Hemangiosarcoma	17.7	42	17,082
9	Miniature schnauzer	10	Male	2	Hemangiosarcoma	22.6	116	4,413
10	Papillon	15	Spayed female	2	Hemangiosarcoma	18.9	31	2,870
11	Labrador retriever	8	Male	2	Hemangiosarcoma	35.1	37	3,929
12	Mongrel	8	Male		Hematoma	23.5	28	1,138
13	Labrador retriever	14	Female		Hematoma	36.1	27	600
14	Maltese	12	Male		Hematoma	30.5	296	735
15	German shepherd	13	Male		Hematoma	39.9	20	1,158
16	Golden retriever	9	Spayed female		Hematoma	30.0	58	1,527
17	Mongrel	12	Castrated male		Hematoma	21.6	23	1,342
18	Welsh Corgi	11	Male		Hemangioma	32.9	65	5,132
19	Chihuahua	16	Male		Lymphoma	23.0	111	11,950
20	Shih tzu	14	Male		Lymphoma	26.1	31	5,599
21	German shepherd	12	Female		Lymphoma	46.0	314	19,429

HSA, six with hematoma, one with hemangioma and three with lymphoma were enrolled in this study. The mean serum ferritin concentration ± SD was 6,222 ± 4,091 *n*g/m*l* in dogs with HSA, 1,083 ± 354 *n*g/m*l* in dogs with hematoma, 5,132 *n*g/m*l* in the dog with hemangioma and 12,326 ± 6,923 *n*g/m*l* in dogs with lymphoma. The mean normal serum ferritin level of 163 clinically healthy dogs was 789 ± 284 *n*g/m*l* [7]. With the upper limit of the serum ferritin concentration reference range determined to be 1,357 *n*g/m*l* (mean + 2 × SD), all HSA cases exceeded the upper limit (Fig. 1). In contrast, only one of the five dogs with hematoma (No. 16; 1,527 *n*g/m*l*) was above the normal range. However, one of the dogs with hemangioma (No. 18) and three with lymphoma (Nos. 19, 20 and 21) had serum ferritin concentrations exceeding the upper limit of the reference range. Therefore, hyperferritinemia in dogs with splenic masses is a frequent finding, but is not specific and can occur with other malignant or benign diseases.

The detailed mechanism of serum ferritin concentration increase in neoplastic diseases is not known, but it is reported that is likely due to increases of stored iron, liver damage or ferritin release from the tumor [20]. In addition, tumor necrosis factor alpha (TNM-α) and interleukin-1α, another proinflammatory cytokine, transcriptionally induce the H chain of ferritin. This finding suggests that inflammation increases intracellular ferritin synthesis [18]. In addition, HSA is thought to cause erythrocyte shearing and microangiopathic hemolytic anemia (MAHA) [5]. Hemolytic anemia is a reported cause of hyperferritinemia [6].

To examine the relationship between serum ferritin concentration and liver damage, we evaluated the association between serum ferritin concentration and plasma ALT activity, as an indicator of hepatocyte injury [14]. We found no significant correlation between serum ferritin level and plasma ALT activity in dogs with HSA (r=0.317, *P*=0.94) or benign splenic diseases (r=0.126, *P*=0.82) (Fig. 2). Therefore, the increased serum ferritin levels found in these dogs do not appear to derive from hepatocyte injury. Although we did not evaluate the liver or bone marrow iron content as an indicator of body iron storage [4, 13], hemoperitoneum was seen in many cases, which would cause loss of iron from the circulation. Therefore, we do not expect that there was a marked increase in body iron storage, and it is unlikely that iron overload was present.

This study did not reveal the cause of hyperferritinemia in dogs with HSA. Probable causes for hyperferritinemia include inflammation, ferritin release from friable tumor tissue [17] and the relationship between HSA and MAHA, but we did not evaluate these factors in this study.

The physiologic significance of serum ferritin is not well understood, but a recent study reported that extracellular ferritin stimulates the iron-independent proliferation of human breast cancer cell lines [2]. Therefore, hyperferritinemia may influence tumor progression. These findings raise interest in a further study of the relationship between serum ferritin levels and the prognosis of this disease.

In conclusion, hyperferritinemia is not specific for splenic HSA, but may have clinical usefulness as a sensitive test in dogs with splenic masses. One limitation of this study was that most of the dogs with HSA were stage 2 or 3, so we have limited information about serum ferritin levels in the early stages of the disease. However, hyperferritinemia was recognized in both stage 1 cases (No. 2, 7), indicating that serum ferritin level may increase in the early stages of HSA.

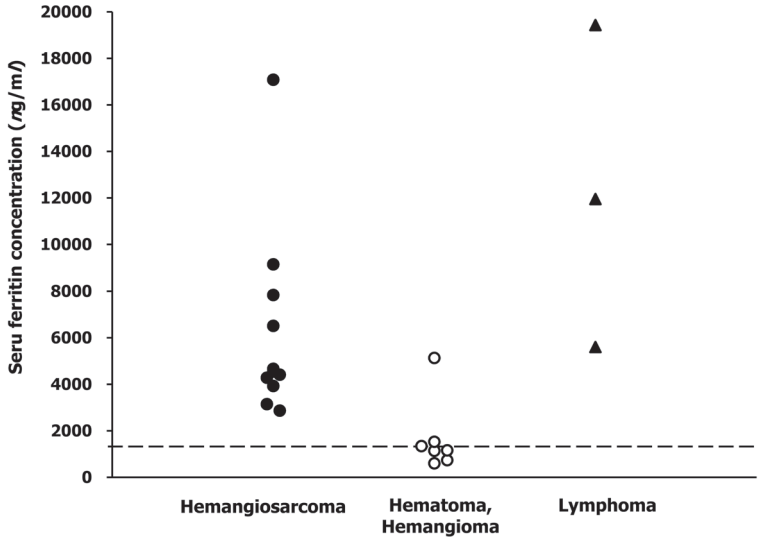

Fig. 1. Serum ferritin concentration in dogs with splenic masses. The dotted line shows the upper limit of the reference value (1,357 *n*g/m*l*), defined from a previous report as mean + 2 × standard deviation.

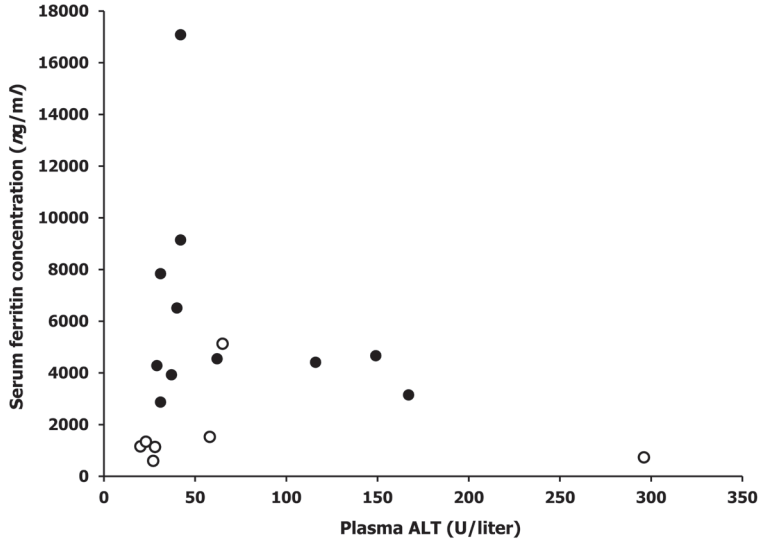

Fig. 2. Correlation between serum ferritin concentration and plasma ALT activity in dogs with splenic hemangiosarcoma (●; r=0.317, *P*=0.94) and benign diseases (○; r=0.126, *P*=0.82). Serum ferritin concentrations were plotted against plasma ALT activity

Splenic HSA is difficult to detect in its early stages, because there are no typical early clinical signs. Clinical signs, such as acute weakness, collapse or hemorrhagic shock, become clear with acute hemorrhage secondary to tumor rupture [17]. Because HSA detected before tumor rupture is reported to have a better prognosis than HSA diagnosed after rupture, early diagnosis and removal of the tumor are essential to achieve a satisfactory prognosis [16]. Splenic HSA is most often diagnosed by radiographic or ultrasound examination. However, because these procedures are not done in typical examinations, HSA might not be found early in cases without any evident clinical signs. Stage 1 disease is not well recognized by dog owners and is therefore difficult to detect. This report deepens our understanding of the subject, and we think that a large-scale study is needed to evaluate whether serum ferritin concentration is useful as a method for the early detection of canine splenic HSA.

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