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Evaluation of Serum Ferritin as a Tumor Marker for Canine Histiocytic Sarcoma

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Background: Canine histiocytic sarcoma (HS) is an aggressive malignancy. Hyperferritinemia has been documented in dogs with HS and could serve as a tumor marker aiding in diagnosis and treatment. In people, hyperferritinemia is found in inflammatory diseases, liver disease, and hemolysis, and thus may occur in dogs with these conditions.

Objective: To determine if serum ferritin concentration is a tumor marker for canine HS.

Animals: Dogs with HS (18), inflammatory diseases (20), liver disease (24), immune-mediated hemolytic anemia (IMHA) (15), and lymphoma (23).

Methods: *Prospective, observational, cohort study*: Serum ferritin concentration was measured at initial diagnosis. Parametric methods were used to compare mean log ferritin concentrations among disease categories. Receiver-operating characteristic curves and likelihood ratios were used to evaluate serum ferritin concentration as a tumor marker.

Results: Varying proportions of dogs with IMHA (94%), HS (89%), liver disease (79%), lymphoma (65%), and inflammatory diseases (40%) had hyperferritinemia. Dogs with IMHA had significantly higher mean ferritin concentration than dogs in all other categories. Dogs with HS had significantly higher mean ferritin concentration than those in the inflammatory disease and lymphoma categories. Mean serum ferritin concentration was not significantly different between dogs with HS and those with liver disease. Decision thresholds were determined to distinguish IMHA and HS from the other diseases associated with hyperferritinemia.

Conclusion: Hyperferritinemia is common in dogs with HS and, after IMHA is ruled out, the degree of hyperferritinemia may be useful in differentiating dogs with HS from dogs with inflammatory diseases, liver disease, and lymphoma.

Key words: Decision threshold; Hepatocyte; Interval likelihood ratio; Receiver-operating characteristic curves.

Histiocytic sarcoma (HS) is an aggressive malignancy of antigen-presenting dendritic cells or phagocytic macrophages.[1,2] HS of dendritic origin occurs as a localized tumor often found on the limbs. Treatment of the localized form involves aggressive surgical excision or amputation, and prognosis is favorable in the absence of metastasis if excision is complete.[1] The disseminated dendritic form arises primarily in the spleen, lung, and bone marrow, with frequent spread to lymph nodes and liver.[1] The macrophage form arises in the splenic red pulp and bone marrow with vascular spread to the liver and lungs.[2] In this form, referred to as hemophagocytic HS, malignant macrophages exhibit marked erythrophagocytic activity resulting in hemolytic anemia and sometimes other cytopenias. Prognosis of metastatic, disseminated, and hemophagocytic HS is poor owing to widespread disease at time of presentation.[1] Chemotherapy with agents such as CCNU has shown some efficacy in slowing progression of disease.[3,4]

Abbreviations:

ALT	alanine aminotransferase
AUC	area under the curve
HS	histiocytic sarcoma
IMHA	immune-mediated hemolytic anemia
KSVDL	Kansas State Veterinary Diagnostic Laboratory
LR	likelihood ratio
ROC	receiver-operating characteristic
TIBC	total iron-binding capacity

Diagnosis of HS presents several challenges. When neoplastic cells are highly pleomorphic, a diagnosis of HS requires differentiation from other sarcomas, round cell tumors, and anaplastic carcinomas.[1] When neoplastic cells exhibit more benign features or do not form discrete masses, HS may be mistaken for reactive histiocytic proliferations.[2] In addition, the clinicopathologic presentation of hemophagocytic HS is very similar to immune-mediated hemolytic anemia (IMHA).[2] Definitive diagnosis of HS requires biopsy with histopathologic evaluation and immunochemical analysis to confirm histiocytic lineage.[1] A serum tumor marker specific for HS would be a valuable diagnostic aid.

A tumor marker is an evaluable molecule that is altered in quantity or quality in the presence of a neoplastic process.[5] It may be used to identify occult neoplasms, determine the tissue of origin, refine prognosis, detect tumor recurrence, or determine response to therapy.[5] Ferritin is an iron storage and acute phase protein made by hepatocytes and cells of hematopoietic origin.[6] In people, hyperferritinemia, unrelated to iron stores, is

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recognized in several malignancies, including tumors of histiocytic origin.[6–11] Hyperferritinemia has been documented in several malignancies of dogs, particularly HS and lymphoma.[a,12,13] In addition, hyperferritinemia occurs in people with hemolytic disorders and liver disease.[11,14] Hyperferritinemia, therefore, may be anticipated in dogs with inflammatory disease, hemolysis, liver disease, and hematologic malignancies, including HS. All of these may be differential diagnoses in dogs with HS based on clinical presentation and initial diagnostic evaluation.

The goal of this study was to determine the diagnostic utility of serum ferritin concentration as a tumor marker to differentiate dogs with HS from dogs with inflammatory disease, liver disease, IMHA, and lymphoma. We hypothesized that dogs with HS would have hyperferritinemia and that the degree of hyperferritinemia would be significantly greater than that in dogs with the other disorders. We utilized receiver-operating characteristic (ROC) curves to assess ferritin as a tumor marker in HS and to select optimal decision thresholds for the differential diagnosis of HS. We also measured serum iron concentration, total iron-binding capacity (TIBC), and ceruloplasmin concentration to determine the independence of ferritin from other measures of iron status and the acute phase response. This study also served to document hyperferritinemia in dogs with liver disease and IMHA.

Materials and Methods

Case Selection

All dogs were examined at the University of Wisconsin Veterinary Medical Teaching Hospital (VMTH) between January 1997 and June 2004, except for 1 dog with IMHA that was examined at a private veterinary clinic. Dogs were included in the study if they had a CBC, serum biochemical profile, a definitive diagnosis, and serum available from initial examination for additional tests (ferritin, serum iron, TIBC, and ceruloplasmin). Dogs were excluded if a definitive diagnosis was not obtained. Sex, breed, and age at diagnosis were recorded for all dogs. Dogs with HS were identified based on cytologic or histopathologic evaluation of tissues collected ante- or postmortem. Definitive determination of histiocytic origin was based on the $CD3^-/CD79a^-/CD18^+$ immunophenotype of the tumor cells.[1] Tumors that were $CD3^-/CD79a^-/CD18^-$ also were stained with MHC-II as another histiocytic marker, or with Melan A or both to rule out amelanotic melanoma. Histiocytic origin was supported by $MHC\text{-}II^+/Melan\ A^-$ reactivity.[1,15,16] Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissues in all cases but 1. In that case, immunocytochemical staining was performed on fresh tissue aspirates owing to the absence of fixed tissue. Dogs with primary inflammatory diseases were identified by the observation of inflammation in tissue samples, on the basis of the known pathophysiology of the definitive clinical diagnosis or both. Dogs with liver disorders had evidence of hepatocellular injury and cholestasis on serum biochemical profile, and all underwent liver biopsy for determination of cause. Dogs with IMHA were identified based on the presence of regenerative anemia with one or more of the following: numerous spherocytes, agglutination, or a positive Coombs' test. Dogs with lymphoma were identified based on cytologic or histopathologic evaluation of tissues collected ante- or postmortem. Staging of lymphoma was based on CBC, serum biochemical profile, urinalysis, cytologic evaluation of lymph nodes, thoracic and abdominal radiographs or ultrasonographic examination or both, and bone marrow aspiration cytology and followed the modified World Health Organization system for canine lymphoma.[17]

Laboratory Testing

CBCs were performed on an impedance electronic cell counter[b] or a laser hematology analyzer.[c] Biochemical profiles were performed on either a dry[d] or wet[e] chemistry analyzer. Results for 1 CBC (inflammatory disease) and 3 biochemical profiles (HS, inflammatory disease, IMHA) were performed elsewhere on unspecified analyzers because the laboratory results were supplied by the referring veterinarians. Serum samples for measurement of ferritin, serum iron, TIBC, and ceruloplasmin were stored at $-70^\circ C$ until they were sent in batches to Kansas State Veterinary Diagnostic Laboratory (KSVDL; Manhattan, KS) for analysis. Canine ferritin concentration was measured by sandwich ELISA technique with mouse monoclonal anti-canine ferritin antibody raised against purified canine hepatic ferritin as described previously.[18] Serum iron concentration and TIBC were measured coulometrically.[19] Ceruloplasmin was measured by the *p*-phenylenediamine oxidase method.[20] Reference intervals for serum ferritin concentration (80–800 ng/mL), serum iron concentration (33−147 μg/dL), TIBC (282–386 μg/dL), and serum ceruloplasmin concentration (< 13.3 mg/dL) were established at the KSVDL. Laboratory personnel at KSVDL did not know the final diagnoses at the time of testing for these variables.

Immunochemical Analysis

Tissue sections, and cytologic specimens in 1 case, were stained immunochemically with antibodies to CD18,[f] CD3,[g] CD79a,[h] MHC-II,[i] and Melan A[j] by the avidin-biotin-peroxidase method. Endogenous peroxidase- and protein-blocking steps preceded application of primary antibodies. Antigen retrieval for tissue sections was performed with steam heat. Canine lymph node and canine kidney served as a positive and negative tissue control for leukocyte antibodies, respectively. A confirmed canine melanoma was used as a positive control for Melan A. For negative antibody controls, buffer was substituted for the primary antibody.

Statistical Analysis

The distributions of ferritin, serum iron, TIBC, and ceruloplasmin within each disease category were assessed for goodness-of-fit by the Shapiro-Wilks statistical test. Distributions were non-Gaussian for serum iron and ferritin in all disease categories. Natural log transformation achieved a Gaussian fit for serum iron and ferritin, and descriptive statistics for these variables are reported in log-transformed units. Ceruloplasmin and TIBC did not require transformation. Differences among the means of the log serum ferritin variable for different disease categories were assessed by analysis of variance followed by the Bonferoni adjustment for multiple pairwise comparisons. Pearson's correlation coefficients were determined among log-transformed values of ferritin, serum iron, TIBC, and ceruloplasmin within each disease category. The above analyses were conducted by statistical software.[k] Nonparametric ROC curves were created to determine optimal decision thresholds for ferritin as a diagnostic test for each disease compared with a previously reported reference healthy population.[18] Subsequently, optimal decision thresholds and intervals for ferritin concentration were examined by likelihood ratio (LR) and the percentage of correctly classified cases to formulate clinical decision rules for the interpretation of ferritin test results compared among the 5 disease groups.[21,22] ROC curve analyses were conducted by statistical software.[l]

Results

Animals

Samples were collected at initial presentation for the primary complaint, but the duration of illness and treatments received before referral or presentation to the VMTH were not recorded.

HS

Thirty dogs with probable HS were identified, but 12 cases were excluded because tissue was unavailable for immunochemical confirmation or was inconclusive for histiocytic origin. Seventeen cases were consistent with dendritic origin based on presentation and histomorphology. Presumptive hemophagocytic variant was diagnosed in 1 dog based on clinical presentation and histologic pattern. These 18 dogs included 9 spayed females, 3 intact females, 4 neutered males, and 2 intact males with a median age of 8.5 years (range, 3.3–12 years). The following breeds were represented: Rottweiler (4), Flat-Coated Retriever (3), Golden Retriever (3), Bernese Mountain dog (2), Labrador Retriever (2), Collie cross (2), Pit Bull Terrier (1), and Miniature Schnauzer (1). Eighty-nine percent (16/18) of dogs with HS had hyperferritinemia. The dog with presumptive hemophagocytic HS had the highest ferritin concentration (20,117 ng/mL) as well as the lowest PCV (17%; reference interval, 37–55%) and highest alanine aminotransferase (ALT) activity (>4,000 U/L; reference interval, 15–84 U/L) secondary to hemolysis and hepatic necrosis, respectively.

Inflammatory Disease

Twenty-two dogs with inflammatory disease were identified. Two were excluded owing to mild or localized inflammatory processes because an acute phase response was not anticipated. Final diagnoses in the remaining 20 dogs included blastomycosis (9), pancreatitis (3), pneumonia (2), septic pyothorax (2), pemphigus foliaceous (1), intestinal intussuseption (1), endocarditis (1), and severe enteritis (1). This group included 4 spayed females, 5 intact females, 8 neutered males, and 3 intact males with a median age of 6.1 years (range, 0.2–13.6 years). The following breeds were represented: Labrador Retriever (3), Golden Retriever (2), and 1 dog each of the following breeds: Akita, Australian Shepherd, Border Terrier, Bulldog, Chow Chow, Doberman Pinscher, Fox Terrier, German Shepherd dog, Old English Sheepdog, Portuguese Water dog, Siberian Husky, St Bernard, Collie cross, Border Collie cross, and mixed breed. Forty percent (8/20) of dogs with inflammatory disease had hyperferritinemia.

Liver Disease

Twenty-seven dogs with liver disease were identified. Three were excluded owing to mild vacuolar degeneration in the absence of additional changes in the liver biopsy. Histologic diagnoses in the remaining 24 dogs included various degrees and combinations of vacuolar degeneration, chronic active hepatitis, hepatocellular degeneration, and suppurative and nonsuppurative inflammation with or without necrosis, cirrhosis or both. One dog had severe hepatocellular necrosis secondary to massive acute hemorrhage from an invasive adrenal carcinoma. The liver category included 13 spayed females, 10 neutered males, and 1 intact male with a median age of 9.5 years (range, 0.6–12.8 years). The following breeds were represented: Doberman Pinscher (3), Beagle (2), German Shepherd dog (2), Labrador Retriever (2), mixed breed (2), and 1 dog each of the following breeds: Bull Terrier, Golden Retriever, Keeshond, Miniature Schnauzer, Parson's Jack Russell Terrier, Pomeranian, Shetland Sheepdog, Standard Poodle, Standard Schnauzer, and Dachshund cross. Seventy-nine percent (19/24) of dogs with liver disease had hyperferritinemia. The dog in this group with the highest ferritin concentration (18,501 ng/mL) had moderate lymphosuppurative hepatitis with individual hepatocyte necrosis and severe bridging fibrosis with pronounced bile ductular proliferation. Four dogs with more widespread necrosis had ferritin concentrations ranging from 2,212 to 7,414 ng/mL, which included the dog with the invasive adrenal tumor.

IMHA

Sixteen dogs with IMHA were identified. Ten of 16 dogs were either Coombs' positive or had agglutination that did not clear with saline dilution. In the remaining 6 dogs, the diagnosis was based on the presence of a regenerative anemia with numerous spherocytes. The IMHA group included 8 spayed females, 1 intact female, and 6 neutered males with a median age of 7.5 years (range, 3.3–11.1 years). The sex of 1 dog, age of 1 dog, and breed of 1 dog were unknown. The following breeds were represented: Cocker Spaniel (3), English Cocker Spaniel (2), and 1 dog each of the following breeds: Airedale Terrier, English Springer Spaniel, Field Spaniel, German Shorthair Pointer, German Shepherd dog, Golden Retriever, Miniature Schnauzer, Standard Poodle, Terrier cross, and mixed breed. Ninety-four percent (15/16) of dogs with IMHA had hyperferritinemia. Serum was markedly hemolyzed in 1 dog, and TIBC and serum iron results were excluded from calculation of descriptive statistics for these variables and for correlations owing to interference with test methodology. These results were identified as outliers during initial statistical analysis.

Lymphoma

Twenty-three dogs with lymphoma were identified. Three were leukemic based on examination of a peripheral blood smear. Of 17 dogs with complete staging, 2 had stage I, 1 had stage II, 4 had stage III, 1 had stage IV, and 9 had stage V lymphoma. The lymphoma category included 6 spayed females, 14 neutered males, and 3 intact males with a median age of 8 years (range, 4–13.9 years). The following breeds were represented: Golden Retriever (4), Labrador Retriever (2), Cocker Spaniel (2), and 1 dog each of the following breeds: Bernese

Table 1. ALT activity and PCV for each disease category.

	ALT (U/L)			PCV (%)		
Disease	Mean	Median	Min−Max	Mean	Median	Min−Max
HS	275[a]	41[a]	15–4,000[a]	39	40	17–55
Inflammatory disease	75	38	18–352	41	42	13–54
Liver disease	978	690	125–3,149	43	44	21–51
IMHA	223	78	15–1,777	18	18	10–29
Lymphoma	173	61	22–880	39	39	29–53

ALT, alanine aminotransferase; HS, histiocytic sarcoma; IMHA, immune-mediated hemolytic anemia; min, minimum; max, maximum.
[a]ALT activity was >4000 U/L in 1 dog, but a value of 4,000 was used for descriptive purposes.

Mountain dog, Boxer, Collie, Corgi, Dachshund, German Shepherd dog, Gordon Setter, Norwegian Elkhound, Rottweiler, Shar Pei, Golden Retriever cross, Afghan cross, Akita cross, Hound cross, and mixed breed. Sixty-five percent (15/23) of dogs with lymphoma had hyperferritinemia.

Laboratory Results

The CBC and clinical chemistry data (not shown) were used for inclusionary purposes and to assist in making the final diagnosis. Because hepatocellular injury and shortened erythrocyte life span may occur in several of these diseases and may contribute to increased serum ferritin concentration, ALT activities and PCVs for each disease category are provided for comparison (Table 1). Figure 1A illustrates the distribution of ferritin results in each disease category. The IMHA group had the highest mean ferritin concentration and this was significantly different from mean ferritin concentration in all other disease categories (Fig 1B). Mean ferritin concentration of the HS category was significantly higher than that in the inflammation and lymphoma groups, but did not differ from mean ferritin concentration of the liver disease group (Fig 1B). Ferritin, serum iron, and ceruloplasmin concentrations and TIBC results within each disease category are presented in Table 2. A positive correlation between ferritin and serum iron concentrations was observed in dogs with lymphoma (0.678, $P = .002$). No other significant correlations were found between ferritin and measures of iron status or acute phase response in any other disease category.

ROC Curves and LR

ROC curves were examined to assess diagnostic utility and to select optimal decision thresholds. When ferritin concentration for each disease category was compared with a healthy reference population reported previously,[18] the area under the curve (AUC) was >0.90 for all categories of disease except inflammatory diseases which had an AUC of 0.86 (data not shown). The ferritin decision threshold with the highest percentage of correctly classified cases was 800 ng/mL, confirming the previously identified upper reference limit for healthy dogs. However, this decision threshold was not useful in differentiating HS from the other disorders.

To more accurately reflect the decision-making process in a clinical situation, we constructed ROC curves that placed dogs with HS in 1 group and dogs with other diseases in a 2nd group—separately or in combination. Overall diagnostic accuracy of ferritin concentration as a diagnostic test for HS assessed by the AUC varied from low ($0.5 < AUC \leq 0.7$) to moderate ($0.7 < AUC \leq 0.9$) (Table 3). Optimal decision thresholds varied depending on which disease was examined in reference to HS (Table 3).

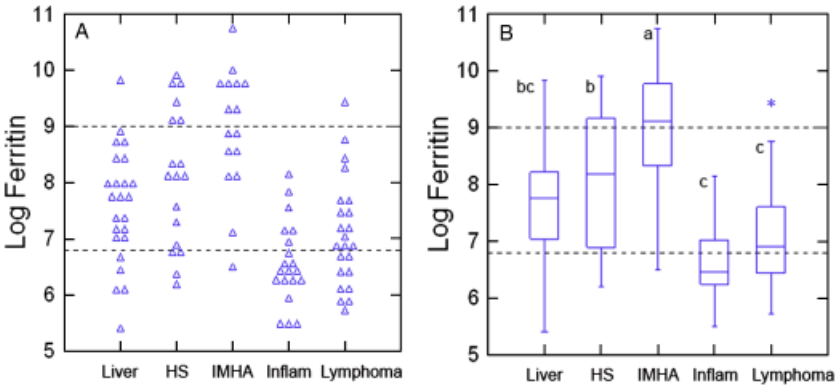

Figure 1. Dot plot (**A**) and box-and-whisker plot (**B**) of log ferritin concentration in dogs with liver disease (Liver), HS, IMHA, inflammatory disease (Inflam), and lymphoma. The lower dashed line represents the upper limit of the reference interval for healthy dogs (800 ng/mL). The upper dashed line represents the upper decision threshold (7,200 ng/mL). In (B) the upper and lower limits of the box reflect the 25th and 75th percentiles. The horizontal line in the box indicates the median. The whiskers represent results within 1.5× the interquartile (IQ) range. Results >1.5× and ≤3× the IQ range are represented by an asterisk. Disease categories that do not share a letter are significantly different from one another (Bonferoni adjustment for multiple pair-wise comparison, $P < .05$).

Table 2. Descriptive statistics for 4 variables in each disease category.

Variable	Disease	n	Mean ± SD	Median	Min–Max	10th–90th Percentile
Ferritin[a] log ng/mL RI 4.38–6.68	HS	18	8.12 ± 1.22	8.18	6.20–9.91	6.47–9.78
	Inflammatory disease	20	6.58 ± 0.73	6.46	5.49–8.16	5.53–7.69
	Liver disease	24	7.60 ± 1.02	7.75	5.41–9.83	6.11–8.75
	IMHA	16	8.94 ± 1.11	9.11	6.50–10.74	7.21–9.98
	Lymphoma	23	7.10 ± 0.96	6.92	5.73–9.44	5.89–8.50
Serum iron[a] log µg/dL RI 3.50–4.99	HS	18	4.35 ± 0.92	4.25	2.20–5.60	3.11–5.55
	Inflammatory disease	20	4.01 ± 0.67	4.09	2.64–5.27	3.13–4.91
	Liver disease	24	4.94 ± 0.85	5.01	1.76–6.04	4.31–5.86
	IMHA	15[b]	5.23 ± 0.72	5.48	3.83–6.24	4.11–6.00
	Lymphoma	23	5.00 ± 0.61	4.84	3.39–6.06	4.25–5.74
TIBC µg/dL RI 282–386	HS	18	303 ± 89	306	137–453	202–401
	Inflammatory disease	20	243 ± 56	242	148–345	170–315
	Liver disease	24	372 ± 107	362	185–628	246–493
	IMHA	15[b]	380 ± 84	371	352–561	285–483
	Lymphoma	23	341 ± 79	368	127–458	235–436
Ceruloplasmin mg/dL RI < 13.3	HS	18	6.4 ± 1.9	6.2	3.7–9.9	3.4–8.8
	Inflammatory disease	20	7.3 ± 2.5	6.9	2.6–11.7	4.3–10.9
	Liver disease	24	6.7 ± 2.8	6.5	2.6–13.6	3.6–10.7
	IMHA	16	8.1 ± 1.9	7.9	5.4–13.0	5.9–9.9
	Lymphoma	23	5.6 ± 1.6	5.4	3.1–8.8	3.8–7.8

HS, histiocytic sarcoma; IMHA, immune-mediated hemolytic anemia; min, minimum; max, maximum.
[a]Ferritin and serum iron concentrations are reported in log values.
[b]One sample was excluded owing to markedly hemolyzed serum.

If all other diseases were combined, the AUC was low (0.654), and the optimal decision threshold was impractically high (16,000 ng/mL). Because dogs with HS and IMHA had some of the highest ferritin concentrations, we combined these 2 categories and compared diagnostic utility of ferritin in discriminating between dogs with HS or IMHA and dogs with the other 3 disorders (Table 3). The AUC of 0.802 indicates ferritin is a moderately useful test for differentiating between these 2 categories of disease with an optimal decision threshold of 7,200 ng/mL.

Post-test odds of disease are significantly increased when positive LR are ≥10, negative LR are ≤0.1, or both.[21] Because positive and negative LR were <10 and >0.1, respectively, knowledge of ferritin concentration only marginally improved the post-test odds of a diagnosis HS when compared with the other diseases (Table 3). However, when dogs with HS and IMHA were combined, the positive LR of 9.9 indicated a substantial increase in post-test odds of HS or IMHA in dogs with ferritin values ≥7,200 ng/mL. Because the degree of hyperferritinemia improves diagnostic utility in dogs with these diseases, we also examined interval LR (Table 4). We determined the likelihood of a diagnosis of HS or IMHA in dogs with normal (≤800 ng/mL), moderately increased (800 < ferritin <7,200 ng/mL), and markedly increased (≥7,200 ng/mL) ferritin concentration compared with the other disorders. Because the interval LR was close to 1 (LR, 0.83), moderately increased ferritin concentrations do not substantially increase post-test odds for a specific diagnosis of HS or IMHA compared with the other diseases, but ferritin concentration below the reference interval moderately decreased the post-test odds (LR, 0.23) of HS or IMHA. Dogs with marked hyperferritinemia were more likely to be diagnosed with HS or IMHA than with inflammatory disease, liver disease, or lymphoma (LR, 9.9).

Table 3. Estimates derived from ROC curves examining the utility of serum ferritin concentration for the diagnosis of histiocytic sarcoma.

Categories for Comparison[a]	AUC (95% CI)	Decision Threshold[b]	Correctly Classified (%)	LR+	LR−
HS versus inflammatory disease	0.854 (0.687–.940)	≥2,400	76	6.1	0.4
HS versus lymphoma	0.750 (0.597–.876)	≥7,200	68	7.7	0.7
HS versus liver disease	0.622 (0.456–.764)	≥8,800	67	6.7	0.8
HS versus inflammatory disease, liver disease, IMHA, lymphoma	0.654 (0.552–.745)	≥16,000	78	2.0	0.9
HS, IMHA versus inflammatory disease, liver disease, lymphoma	0.802 (0.711–.875)	≥7,200	78	9.9	0.6

AUC, area under the curve; CI, confidence interval; LR+, positive likelihood ratio; LR−, negative likelihood ratio; HS, histiocytic sarcoma; IMHA, immune-mediated hemolytic anemia; ROC, receiver-operating characteristic.
[a]Disease categories were grouped and compared as indicated.
[b]Decision thresholds for ferritin (ng/mL) were selected based on the highest percentage of correctly classified cases.

Table 4. Interval likelihood ratios for ferritin concentrations in dogs with histiocytic sarcoma or IMHA compared with the other disorders.

Ferritin Concentration (ng/mL)	No. Dogs with HS and IMHA	No. Dogs with Inflammation, Liver Disease, Lymphoma	LR Calculation
≥7,200	15	3	(15/34)/(3/67) = 9.9
7,199–800	16	38	(16/34)/(38/67) = 0.8
<800	3	26	(3/34)/(26/67) = 0.2
Total	34	67	

HS, histiocytic sarcoma; IMHA, immune-mediated hemolytic anemia; LR, likelihood ratio.

Discussion

Our results show that hyperferritinemia is common in dogs with HS and, therefore, that ferritin may be a useful serum tumor marker for this neoplasm. Similar to our study, previous reports also have documented hyperferritinemia in canine lymphoma and HS.[a,12,13] We now show that dogs with liver disease, IMHA, and inflammation also may have hyperferritinemia. In people, some of the highest ferritin concentrations were observed with hemolytic disease and liver necrosis.[23] In our study, the highest mean ferritin concentration was found in dogs with IMHA. In contrast, the dog in the liver disease category with the highest ferritin concentration had necrosis only of low numbers of individual hepatocytes, whereas 4 other dogs with more widespread necrosis had moderate hyperferritinemia. The highest ferritin concentration in the HS category was observed in a dog with both marked hemolytic anemia and marked hepatic necrosis.

Because dogs with hyperferritinemia could be found in all disease categories, ferritin concentration merely above the reference interval does not appear to be a useful discriminatory test for HS. Our results, however, show that different decision thresholds could be applied to aid in the differentiation between HS and inflammatory disease (2,400 ng/mL), between HS and lymphoma (7,200 ng/mL), or between HS and liver disease (8,800 ng/mL). Because both HS and IMHA could result in marked hyperferritinemia, a practical decision threshold to differentiate between HS and IMHA or between HS and all other disease categories could not be identified. Instead, we show that dogs with marked hyperferritinemia (≥7,200 ng/mL) are more likely to have HS or IMHA than any of the other diseases (LR, 9.9). For dogs with marked hyperferritinemia, IMHA usually can be differentiated from HS by the presence of numerous spherocytes, agglutination, a positive Coombs' test or some combination of these.[2,24] The variation in LR determined on an interval basis indicates that consideration of the degree of hyperferritinemia offers improved clinical utility compared with a single decision threshold.[22] Greater sample size for all disease categories would be necessary to determine if narrower intervals would further improve diagnostic utility of mildly to moderately increased ferritin concentrations.

The cause of hyperferritinemia in both neoplastic and non-neoplastic disorders is likely multifactorial. Proposed mechanisms include erythrocyte destruction, alteration in erythropoiesis, release by tissue damage, injury to hepatocytes, and, in the case of malignancies, production by tumor cells.[6,8,10,12,25] In a study of serum ferritin concentrations in people with liver disease, serum ferritin was correlated with the product of liver iron content and ALT activity, suggesting that the degree of hyperferritinemia is related to both hepatic iron content and extent of injury.[14] Increased hepatic iron is recognized in dogs with chronic hepatitis, but to the authors' knowledge, serum ferritin concentrations have not been reported previously in dogs with liver disease.[26,27] Several of these mechanisms may contribute to the hyperferritinemia in canine HS, as demonstrated by the dog with the most marked hyperferritinemia that had HS and both marked hemolysis and marked hepatic necrosis. Additional studies are needed to determine if neoplastic histiocytes synthesize and release ferritin or if ferritin is produced by normal cells in response to the neoplasm, by erythrocyte destruction, tissue injury, hepatocellular damage, or some combination of these factors.

Concurrent with ferritin concentration, we measured serum iron concentration and TIBC in study dogs to ascertain whether changes in ferritin were related to changes in iron status. Previous studies indicate that serum ferritin concentration reflects total body iron stores in healthy dogs and in dogs with iron deficiency.[18,28,29] The lack of correlation between ferritin and other measures of iron metabolism in dogs with HS, inflammatory disease, liver disease, and IMHA suggests that increases in ferritin concentration are not specifically associated with iron status in these diseases. The exception is a moderately positive correlation between serum ferritin and iron concentrations in dogs with lymphoma. Mean serum iron concentration in dogs with lymphoma in this study was near the upper limit of the reference interval. This contrasts with another study in which mean serum iron concentration in dogs with lymphoma was lower than that of healthy dogs.[13] Others have shown a lack of correlation between serum iron and ferritin concentration in people with malignancies, and attributed this to low iron content of ferritin associated with tumors.[11] Mammalian ferritin is a mix of several isoferritins that vary in subunit composition and iron content.[30] Isoferritins can be differentiated by isoelectric focusing and can have different immunologic properties.[31,32]

There was no correlation between ferritin and ceruloplasmin in any disease category. However, serum ceruloplasmin concentration was increased in only 1 dog with liver disease despite evidence of inflammation in many of the study dogs. Although ceruloplasmin has been shown to have high specificity and positive predictive value for inflammation compared with total leukocyte, neutrophil, or band neutrophil counts, it has low to moderate sensitivity and negative predictive value.[33] Alternative acute phase proteins in dogs, including C-reactive protein, serum amyloid A, haptoglobin, and alpha-1 acid glycoprotein, were not measured in our study.[34]

This study has some limitations. Most dogs with HS had advanced disease at the time of presentation, which may have selected for dogs with higher ferritin concentrations. However, dogs with HS typically present late in the course of disease, as has been documented in the literature.[1,2] We did not correlate tumor burden with ferritin concentration, but future studies to answer this question could indicate whether ferritin has prognostic value. All dogs were entered into this study upon initial presentation for the current illness, but onset and duration of illness and previous treatments received at the referring veterinarian were not considered. Prior glucocorticoid exposure can affect serum iron concentration and to a lesser extent TIBC, which may have affected correlations with ferritin.[35] Liver damage in 1 dog resulted from hepatic necrosis secondary to hypoxia after massive internal hemorrhage from an invasive adrenal carcinoma; whether this malignancy contributed to the dog's serum ferritin concentration cannot be determined.

Our results show that hyperferritinemia is common in dogs with HS and that hyperferritinemia can also be detected in dogs with inflammatory disease, liver disease, IMHA, and lymphoma. Therefore, ferritin concentrations above the upper reference interval alone are not diagnostic for HS. The degree of hyperferritinemia, however, is useful, and we have suggested potential decision thresholds or intervals for differentiating HS from inflammation, liver disease, and lymphoma. The post-test probability of HS or IMHA is high if hyperferritinemia is marked. A larger case series is needed to confirm these findings, and additional studies are needed to determine whether ferritin can serve other roles as a serum tumor marker, such as indicating tumor burden (prognosis), response to treatment, and recurrence.

Footnotes

[a] Bush JM, Garrett LD. Serum ferritin in canine malignancies. In: Preceedings of the Veterinary Cancer Society annual meeting, October 11–14, 2001, Baton Rouge, LA (abstract)
[b] Coulter S770 electronic cell counter, Beckman Coulter, Fullerton, CA
[c] Advia 120, Siemens Healthcare Diagnostics, Deerfield, IL
[d] Vitros 250, Ortho Clinical Diagnostics, Johnson & Johnson, Rochester, NY
[e] Hitachi 912, Roche Diagnostics, Indianapolis, IN
[f] FE3.9F2, Leukocyte Antigen Laboratory, Davis, CA
[g] Polyclonal, Dako, Carpinteria, CA
[h] HM57, Dako
[i] HLA-DR TAL.1B5, Dako
[j] A103, Dako
[k] SYSTAT 12 software, SYSTAT Software Inc, San Jose, CA
[l] STATA/MP 10.1 software, StataCorp LP, College Station, TX

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