



Preliminary evaluation of C-reactive protein (CRP) as a prognostic marker in dogs with histiocytic sarcoma

L. Schiavo^{1,3} · A. Giuliano² · T. L. Williams¹ · J. M. Dobson¹

Received: 28 May 2024 / Accepted: 24 December 2024 / Published online: 24 February 2025
© The Author(s) 2025

Abstract

Histiocytic sarcoma (HS) is aggressive neoplasia arising from cells of the dendritic cell or macrophage lineage. Previously recognised negative prognostic factors are the presence of metastasis, anaemia, hypoalbuminemia, and thrombocytopenia. C-reactive protein (CRP) is an acute phase protein (APP), previously investigated as a prognostic marker in different neoplasms in human and veterinary medicine, including in Bernese Mountain dogs with histiocytic sarcoma, however no studies have evaluated serum CRP concentrations in all breeds with histiocytic sarcoma. Our study aimed to evaluate the prognostic value of serum CRP concentrations at the time of presentation and in different stages of the disease in a cohort of dogs, of different breeds, with histiocytic sarcoma. Dogs diagnosed with histiocytic sarcoma that not received any treatment prior to CRP measurement were included in the study. Patients were grouped according to the location of the primary tumour, tumour stage and treatment received (no treatment, localised treatment and systemic treatment). A Mann Whitney U was used to compare the CRP value with the stage and site of the tumour, while a Spearman's correlation coefficient was used to compare the serum CRP with continuous variables. Thirty-five dogs were included. The median survival time for the entire population was 106 days. Increasing serum CRP concentrations ($p = 0.009$) and younger age ($p = 0.003$) were associated with decreased survival time in the multivariable model. No correlation was found between serum CRP concentrations and the stage of the disease ($p = 0.327$) or the site of the primary tumour (visceral vs. periarticular $p = 0.504$). Serum CRP concentrations and age may be additional prognostic factors in patients with histiocytic sarcoma, that are worthy of further investigation in larger studies.

Keywords Acute phase protein · Canine histiocytic sarcoma · C reactive protein · Prognostic factor

Introduction

Canine histiocytic sarcoma (HS) is an aggressive neoplasia arising from the malignant transformation of cells of dendritic or macrophage lineage (Moore et al. 2006). Both of these cells arise from bone marrow-derived CD34⁺ cells, and, under the influence of different cytokines, they differentiate to form cells of the monocyte/macrophage lineage or the dendritic cell (DC) lineage (Fulmer and Mauldin 2007). Histiocytic sarcoma was initially recognised in Bernese Mountain dogs, and now other breeds such as the Flat-coated Retriever and Golden Retriever are considered at increased risk of development of this tumour (Dobson 2013). More recently, pulmonary HS has also been recognised in Pembroke Corgi and Miniature Schnauzer breeds (Purzycka et al. 2020; Lenz et al. 2017). Canine histiocytic sarcoma can present as a localised or a disseminated form, with periarticular locations, lungs, spleen and soft tissues

✉ L. Schiavo
lucaschiavo256@gmail.com;
luca.schiavo@vetspecialists.co.uk

✉ A. Giuliano
a.giuliano@cityu.edu.hk

T. L. Williams
tlw27@cam.ac.uk

J. M. Dobson
jmd1000@cam.ac.uk

¹ Department of Veterinary Medicine, Queen's Veterinary School Hospital (QVSH), University of Cambridge, Cambridge, UK

² Department of Veterinary Clinical Sciences, Jockey Club College of Veterinary Medicine, City University of Hong Kong, Hong Kong, China

³ Davies Veterinary Specialists, part of Linnaeus Veterinary Ltd, Higham Gobion, Hitchin, UK

representing the most common primary sites for localised disease (Cannon et al. 2015; Constantino-Casas et al. 2011). In the localised form, the tumour is usually confined to a single site, whereas the disseminated form usually presents with multiple lesions in two or more distant sites (Kezer et al. 2018). The disseminated form can be the result of a metastatic localised tumour or may represent a primary multicentric disease in which multiple lesions occur simultaneously (Klahn et al. 2011). Even in the localised form, the rate of metastatic disease is up to 70% at the time of death, with loco-regional lymph nodes, lungs, spleen, and liver considered the most common sites of metastasis (Fidel et al. 2006; Skorupski et al. 2009).

Histiocytic sarcoma is considered a fatal disease, with the majority of patients succumbing to the disease within 6 months of diagnosis (Kezer et al. 2018). Hypoalbuminemia, anaemia, thrombocytopenia, disseminated disease, advanced stage, visceral involvement and the use of steroids at the time of diagnosis have been identified as negative prognostic factors (Cannon et al. 2015; Klahn et al. 2011; Dervisis et al. 2017).

The study of acute-phase proteins (APPs) is gaining interest in veterinary medicine, and these represent a group of proteins that are released into the circulation following tissue injury (Nakamura et al. 2008). While originally it was thought that these proteins were produced only by hepatocytes, there is growing evidence that several cell types, including lymphocytes, can produce APPs under the influence of cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor α (TNF α) (Ceron et al. 2005; Yamashita et al. 1994). Their release is independent from the initiating cause of the tissue injury, which could be inflammatory, infectious or neoplastic. (Ceron et al. 2005). C-reactive protein (CRP) was the first described acute phase protein and it has different biological functions including the induction of several cytokines, the inhibition of chemotaxis and the modulation of neutrophil function (Ceron et al. 2005; Murata et al. 2004). CRP is considered the most sensitive acute-phase protein in dogs, and serum concentrations are not influenced by the use of corticosteroids, age and sex unlike other positive APPs such as haptoglobin (Murata et al. 2004; Mukorera et al. 2011). In addition, the concentration of CRP is not influenced by different clinical and pathological variables such as body temperature and white blood cell (WBC) concentration (Nakamura et al. 2008). These characteristics led to the study of CRP in veterinary oncology (Selting et al. 2016, 2015). An increase in serum CRP concentrations was found to be correlated with the development of several neoplasms such as canine lymphoma, oesophageal and mammary tumours (Nivy et al. 2014; Merlo et al. 2007; Planellas et al. 2009), and serum CRP concentrations were also found to be correlated with remission status in patients with lymphoma (Nielsen et al. 2007). Serum

CRP concentrations are elevated in Bernese Mountain dogs with disseminated histiocytic sarcoma compared to a healthy control group (Nikolic Nielsen et al. 2013), however, to the authors' knowledge no previous study has evaluated serum CRP concentrations as a potential biomarker for prognosis or the association of serum CRP concentrations with the stage of disease.

The aim of this study was to evaluate the correlation between serum CRP concentrations in dogs with histiocytic sarcoma with the stage of the disease at presentation, the localisation of the primary tumour and survival time. Our hypothesis was that increasing serum CRP concentrations would correlate with a more advanced stage of the disease and a worse prognosis.

Materials and methods

Medical records from the Queen's Veterinary School Hospital (University of Cambridge), between January 2012 and November 2021 were reviewed for dogs diagnosed with histiocytic sarcoma. Follow up information was retrieved from the medical records of the hospital or by contacting the referring veterinary surgeons. Patients were included if they were diagnosed with histiocytic sarcoma by cytology or by histopathology (+/- immunohistochemistry). Patients which were diagnosed by cytology only were included only if they showed the presence of numerous pleomorphic, large, discrete mononuclear cells with abundant, lightly basophilic, vacuolated, granular cytoplasm and marked anisocytosis and anisokaryosis as described by Brown et al. (Brown et al. 1994).

Serum CRP concentrations at presentation were extracted from the medical history. For patients without recorded serum CRP concentrations, serum CRP concentrations were measured using archived frozen (-80°C) serum samples taken at the time of presentation. Patients were excluded if they received any systemic chemotherapy, surgery, or radiotherapy before serum CRP measurement and if no record of the serum CRP concentration and no stored serum sample was available. Patients who received other treatments such as NSAID, steroids or antibiotics before measurement were not excluded.

Serum CRP concentrations were measured in a single clinical pathology laboratory on an Olympus AU480 biochemistry analyser (Beckman Coulter) using a commercially available assay (immunoturbidimetric CRP assay, Randox), previously validated for use in dogs (Klenner et al. 2010). The laboratory reference interval for CRP was $< 8.2\text{ mg/L}$ and the limit of blank of the assay was 2.2 mg/L .

Based on the primary location of the tumour, patients were divided into peri-articular, visceral, and cutaneous groups. Full staging, consisting of CT scan of thorax and abdomen, or thoracic radiographs and abdominal ultrasound

was required for patients to be included in the study. Based on the imaging results, patients were clinically staged as ‘T’ ‘Localised’ histiocytic sarcoma, a solitary primary tumour with no evidence of loco-regional nodal or distant metastasis was found; ‘T & N’ ‘Lymph node metastasis’ if there was cytological or histological evidence of locoregional lymph node metastasis but with no distant metastasis present; T,N &M ‘Disseminated’, if two or more different sites, excluding the loco regional lymph nodes, were affected. Dogs were divided into 3 groups based on treatment received: patients not receiving any treatment; local treatment (surgery or radiotherapy) only; systemic chemotherapy (+/- local treatment).

Statistical analysis

Statistical analysis was performed using commercially available statistical software (SPSS version 27.0- IBM). For statistical purposes, a serum CRP concentration below the limit of blank of the assay (2.2 mg/L) was assigned an arbitrary value of 1.1 mg/L. Comparison of serum CRP concentrations between the T&N group and the T or TN&M groups, or between the cutaneous localisation and periarticular and visceral sites was not possible due to low numbers of cases in the T and cutaneous involvement groups, hence the Mann Whitney U test was used to compare serum CRP concentrations between groups (based on stage and site). Spearman’s correlation coefficient was used to evaluate associations between continuous variables. Kaplan Meier survival curves were constructed to calculate the median survival times of different groups. Univariable Cox regression analysis was used to evaluate the association between serum CRP concentration, age, stage, site (excluding cutaneous site) and treatment type with overall survival time (all cause mortality). Animals that were alive at the time of writing were censored from the survival analyses. Parameters that showed a tendency towards a significant association with survival time ($P < 0.2$) were included in a multivariable Cox regression model. Data are presented as median [range] unless otherwise specified and $p < 0.05$ was considered statistically significant.

Results

Patients’ characteristics

A total of 74 cases were diagnosed with histiocytic sarcoma during the study period. Of these, 13 cases did not have complete medical records and in 15 cases the serum CRP concentration was not measured at the time of presentation and no archived serum was available. Of the 46 cases remaining, 11 were excluded as they received treatment before the

measurement of the CRP (surgery was performed in 9 cases and chemotherapy in 2 cases). Following exclusion, 35 cases were eligible for inclusion. The Flat-coated Retriever was the most represented breed (23 cases) followed by Bernese Mountain dogs (2), Labrador (2) and Bullmastiff (2). Other breeds included Cairn Terrier (1), Rottweiler (1) Miniature Schnauzer (1), Maltese (1) and German Shepherd (1). Female neutered dogs were the most common (14), followed by male neutered (11), male entire (8) and female entire (2). The median age was 8 years [4 to 12 years] with a median body weight of 32.3 kg [8.1 to 58.6 kg].

Diagnosis

Of the 35 cases included, the diagnosis was achieved by cytology alone in fourteen cases, histology alone in three cases, both cytology and histopathology in three cases, and histopathology and immunohistochemistry in four cases. Cytology, histopathology, and immunohistochemistry combined were utilised for diagnosis in eleven patients. The methods of diagnosis is summarised in Table 1.

Lymph node sampling was performed in 19 cases. In 16 cases fine needle aspiration of the lymph node was performed. In the remaining 3 patients, the lymph node was evaluated by histology. Lymph node metastases were found in 8 cases. Of these, 6 patients were also diagnosed with distant metastatic disease.

All patients underwent full clinical staging. Three view thoracic radiographs and abdominal ultrasound were performed in 20 cases, while computed tomography (CT) was performed in 15. The tumour stage was classified as localised (T) in 16 cases (12 periarticular, 3 visceral and 1 cutaneous localisation), lymph node metastasis (T&N) in 2 (both with periarticular localisation) and disseminated (TN&M) in 17 cases (10 periarticular, 6 visceral and 1 cutaneous localisation). The tumour location was classified as periarticular in 24 cases, visceral in 9 cases and cutaneous in 2. Group size and characteristics were summarised as Table 2. Selected patient characteristics, including treatment received, are summarised in Table 3.

Treatment and survival

The median survival time for the whole population was 106 days [2 to 400 days; mean 120 days]. At the time of writing the study 32 of the 35 patients had died and three were alive at 122, 148 and 235 days post-diagnosis. Death or euthanasia was attributable to the tumour in all cases that had died.

Based on treatment, 8 patients were classified in the no-treatment group, 13 in the localised treatment group (3 had surgery and 10 radiation therapy), and 14 in the chemotherapy group (6 received additional local treatment). The patients treated with radiation therapy received

Table 1 Methods of diagnosis in the population examined. 14 patients were diagnosed with cytology alone, 3 with histology alone, 3 with histology and cytology, 4 with histology and immunohistochemistry and 11 with cytology, histology and immunohistochemistry

CASE	CYTOLOGY	HISTOLOGY	IMMUNOHISTOCHEMISTRY
1		x	X
2	X		
3	X		
4	X		
5		X	
6		X	X
7		X	X
8	X	X	X
9	X		
10	X	X	X
11	X	X	X
12	X		
13	X	X	X
14	X	X	X
15	X		
16	X		
17	X	X	
18		X	
19		X	X
20	X		
21	X		
22		X	
23	X		
24	X	X	X
25	X		
26	X		
27	X	X	X
28	X	X	
29	X	X	X
30	X		
31	X	X	X
32	X	X	
33	X		
34	X	X	X
35	X	X	X

a hypofractionated, manually planned protocol. The median total dose delivered was 34 Gy [17–34 Gy] with a median of 4 (Fulmer and Mauldin 2007; Dobson 2013; Purzycka et al. 2020; Lenz et al. 2017) fractions. The first-line chemotherapy used in all treated patients was lomustine (Bova Specials UK) given every three weeks. Median dose administered was 65 mg/m² [60–70 mg/m²] with a median of 5 (Moore et al. 2006; Fulmer and Mauldin 2007; Dobson 2013; Purzycka et al. 2020; Lenz et al. 2017; Cannon et al. 2015;

Constantino-Casas et al. 2011; Kezer et al. 2018) treatments administered. Rescue protocols were used in 2 cases, toceranib phosphate was used in 1 case and doxorubicin in another patient. The choice of the rescue protocol was based on the clinician preference.

Association between serum CRP concentration and tumour site or tumour stage

Median serum CRP concentration for the entire population was 9.1 mg/L [< 2.2–65.4 mg/L]. CRP was measured at the time of presentation in 29 cases and on fresh frozen serum in the remaining six cases. The median time from sample collection to CRP analysis, in these six cases, was 1648 days [834 to 2848 days]. CRP was above the reference interval in 18 of the 35 cases (51%).

Serum CRP concentration did not differ between dogs with periarticular disease and dogs with visceral disease (7.8 [< 2.2–51.9] mg/L vs. 9.1 [5.3–65.4] mg/L; $p=0.504$). The two dogs with cutaneous involvement had a serum CRP concentration of 15.5 mg/L and 3.2 mg/L.

Serum CRP concentration in dogs with disseminated disease and dogs with localised disease were not significantly different (5.8 [< 2.2–65.4] mg/L vs. 14.5 [< 2.2–24.8] mg/L; $p=0.327$). Dogs with lymph node metastasis ($n=2$) had serum CRP concentrations of 13.9 mg/L and 14.7 mg/L.

Parameters associated with survival time

In the univariable analysis, increasing serum CRP concentration was associated with a decreased survival time (hazard ratio (HR) = 1.022, 95% confidence interval (CI) = 1.000–1.044; $p=0.049$). Patients presenting with a visceral location of the tumour did not have a different survival time than dogs with a periarticular location of the tumour (MST 66 [2 to 246] days vs. 115 [2 to 400] days, HR 1.326 [95% CI 0.573–3.068]; $P=0.51$). The two dogs with a cutaneous tumour had an MST of 6 and 30 days. Dogs with localised disease (T group) (MST 115 [30–400] days) and dogs with disseminated disease (TN&M) (MST 67 [2–69] days) did not have significantly different survival times ($P=0.327$). Dogs in the lymph node metastasis group had MSTs of 35 and 137 days. Surprisingly, increasing age was associated with an increased survival time (HR 0.78 [95% CI 0.649–0.938]; $P=0.008$). Patients receiving localised (106 [6–400] days, $n=13$) or systemic treatment (145 [3–268] days, $n=14$) tended to show a better prognosis compared to those receiving no treatment (41 [2–148] days, $n=8$), however, in both groups this did not reach statistical significance (localised treatment vs. no treatment: HR 0.406 [95% CI 0.149–1.107]; $P=0.078$; systemic treatment vs. no treatment: HR 0.427 [95% CI 0.161–1.1132]; $P=0.087$, Fig. 1).

Table 2 Group size and characteristics based on stage and primary localisation of the disease

	GROUPS	NUMBER OF PATIENTS
POPULATION		35
	PRIMARY LOCALIZATION	24
STAGE	Visceral	9
	Cutaneous	2
	T (Localised disease)	16 (12 periarticular – 3 visceral – 1 cutaneous)
	T&N (Primary tumour and lymph node)	2 (2 periarticular)
	TN&M (Presence of distant metastasis)	17 (10 periarticular—6 visceral – 1 cutaneous)

Age, serum CRP concentration, and treatment type were included in a multivariable Cox regression analysis. The two dogs with a cutaneous tumour were excluded from the multivariable analysis due to low numbers of cases in this group. In the final model, decreasing age (HR 0.744, 95% CI 0.611–0.905; $P=0.003$) and increasing serum CRP concentrations (HR 1.033, 95% CI 1.008–1.059; $P=0.009$) were both independently associated with shorter survival time.

Discussion

In this study, we evaluated the association between the serum CRP concentration and several clinical features, in patients with histiocytic sarcoma. In our cohort of patients, increasing serum concentration of CRP was associated with a reduced survival time in patients with this malignancy in the univariable and multivariable analysis. These results correlate with previous findings in canine mammary tumours as well as in different haematological malignancies in which serum CRP concentrations were associated with several negative prognostic factors and survival ((Saellström et al. 2022; Tecles et al. 2009)). Cancer-associated inflammation has continuously gained interest during the past few decades, and several links between the cancer inflammatory population and the mechanism necessary for tumour remodelling and neoangiogenesis were previously described (Balkwill and Mantovani 2001; Mantovani et al. 2008). The increase in the serum CRP concentration observed in many dogs (51%) with histiocytic sarcoma could reflect a stronger cancer-related inflammatory response and production of IL-6 as a direct stimulator of CRP production (Nikolic Nielsen et al. 2013; Dethlefsen et al. 2013; Raposo et al. 2015). Alternatively, a marked increase in serum CRP concentrations could reflect a higher degree of tumour necrosis, another previously recognised marker of aggressiveness in some canine cancer (Kuntz et al. 1997; Pradel et al. 2018),

however, associations between serum CRP concentrations and tumour necrosis were not evaluated as part of the present study. Interestingly, in our study, the serum CRP concentration did not correlate with either tumour stage, or tumour localisation. Serum CRP concentration has been demonstrated as a potential marker of prognosis in canine mammary tumours, and in some cases it is correlated with the tumour grade, however, its correlation with the stage of mammary neoplasia is still controversial (Sylman et al. 2018). Previous literature in humans showed that serum CRP concentration was correlated with survival time in patients with localised disease, while no correlation was found in patients with metastatic disease (Allin et al. 2009). The same study suggested that serum CRP concentration could potentially reflect a general clinical impact of the tumour on the patient rather than reflecting specifically the stage of the disease (Allin et al. 2009). Specifically in our study, it cannot be completely excluded that the use of different staging methods could have potentially led to an underestimation of the extent of disease in some patients staged by thoracic radiographs and abdominal ultrasound alone.

The primary location of the tumour has previously been reported as a potential prognostic factor in canine patients with histiocytic sarcoma. Previous studies showed that patients with periarticular tumours had a longer survival, compared to patients presenting with visceral tumours (Klahn et al. 2011; Skorupski et al. 2009, 2007). A previous study showed that the survival of patients with periarticular tumours was significantly longer than in patients with non-periarticular tumours with a median survival time of 391 days compared to 128 days respectively (Klahn et al. 2011). In the present study, the survival time of dogs with visceral localisation was not statistically different to that of periarticular tumours. This may reflect the relatively small sample size or differences in treatment administered.

In our study the stage of the disease, was not associated with prognosis in histiocytic sarcoma. This is in contrast

Table 3 Patient characteristics at presentation, including primary localisation of the disease, serum C Reactive Protein (CRP) at presentation and treatment received

Case	Localisation	Stage	Serum CRP mg/l	Treatment
1	stifle	disseminated	14.9	Chemotherapy and radiotherapy
2	temporomandibular joint	localised	4.2	Radiotherapy
3	shoulder	disseminated	51.9	Radiotherapy
4	left carpus	Lymph node	14.7	Palliative
5	elbow	localised	4.2	Radiotherapy
6	Liver	disseminated	9.1	Palliative
7	elbow	localised	23.2	Palliative
8	shoulder	localised	< 2.2	Chemotherapy
9	shoulder	disseminated	6	Radiotherapy
10	pelvis	localised	24.8	Radiotherapy
11	liver	disseminated	31.9	Chemotherapy
12	lung	disseminated	6.6	Chemotherapy
13	shoulder	disseminated	< 2.2	Radiotherapy
14	spleen	localised	7.7	Surgery
15	skin flank	disseminated	15.5	Chemotherapy
16	shoulder	disseminated	< 2.2	Chemotherapy and radiotherapy
17	shoulder	localised	47	Radiotherapy
18	kidney	localised	5.3	Surgery
19	elbow	disseminated	< 2.2	Chemotherapy
20	shoulder	localised	9.4	Radiotherapy
21	scapula	lymph node	13.9	Chemotherapy
22	spleen	disseminated	65.4	Surgery
23	spleen	disseminated	17.9	Palliative
24	scapula	disseminated	14.5	Palliative
25	shoulder	disseminated	40.8	Palliative
26	elbow	Localised	9.6	Radiotherapy
27	left submandibular skin	Localised	3.2	Palliative
28	scapula	Disseminated	1.1	Chemotherapy and radiotherapy
29	stifle	Localised	6.3	Radiotherapy
30	shoulder	Disseminated	16.5	Chemotherapy
31	elbow	Localised	1.1	Chemotherapy and radiotherapy
32	elbow	Localised	1.1	Palliative
33	liver	Disseminated	6.1	Chemotherapy
34	Carpus	Localised	1.1	Chemotherapy and radiotherapy
35	Spleen	Localised	43.9	Chemotherapy and surgery

with part of the previous literature regarding histiocytic sarcoma, where stage was considered as a factor associated with prognosis (Dervisis et al. 2017). In a previous study, patients with disseminated disease had a shorter survival time, 78 days, compared to patients with localised form of the disease, 398 days. In addition, patients with disseminated forms of HS were nearly three times more likely to die due to the disease. In the same study, the presence of lymph node metastasis was a negative prognostic factor in patients with localised forms of the disease (180 vs 461 days) (Dervisis et al. 2017). In addition, patients dogs with periarticular forms of the disease and concurrent metastasis have short prognosis compared to patients with no metastases (980

vs 253 days) (Klahn et al. 2011). As previously discussed, the difference in staging methods, and the low number of patients, in our study could have caused misclassification of some patients and reduced the statistical power of the study.

In the population examined, age was inversely correlated with the survival time. To our knowledge, this has not previously been reported as a prognostic marker for patients with histiocytic sarcoma. In a previous study analysing clinical and prognostic factors in patients with histiocytic sarcoma, Bernese Mountain dogs were younger at the time of diagnosis compared to other breeds, in particular Retrievers, but prognostic significance of age was not reported (Dervisis et al. 2017). In our population, there were only two Bernese

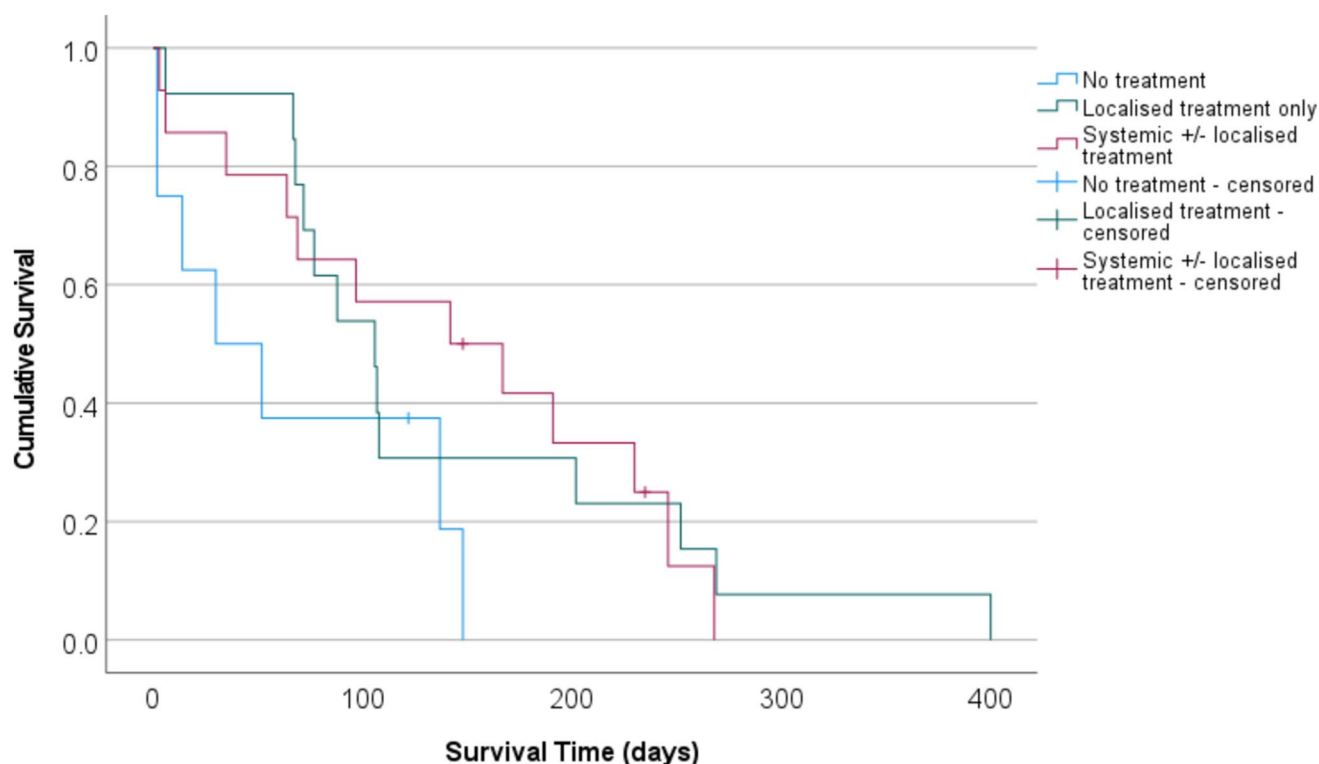


Fig. 1 Kaplan–Meier curve showing the association between survival time and treatment. On univariable Cox regression analysis, patients receiving localised treatment localised (106 [6–400] days, $n=13$) or systemic treatment (145 [3–268] days, $n=14$) had longer MST compared to patients receiving no treatment (41 [2–148] days, $n=8$).

However, this was not statistically significant (localised treatment vs. no treatment: HR 0.406 [95% CI 0.149–1.107]; $P=0.078$; systemic treatment vs. no treatment: HR 0.427 [95% CI 0.161–1.1132]; $P=0.087$)

Mountain dogs although they were both younger than the median age of the population (4 and 6 years), however due to this low number of this breed it seems unlikely that the association between younger age and worse survival was a reflection of breed alone. In human medicine, young age has been found to be an independent negative prognostic factor in patients with histiocytic disorders in several reports (Satter and High 2008), with patients younger than 2 years more likely to develop a multicentric form of the disease (with lung involvement) and thrombocytopenia (a recognised negative prognostic factor in humans and dogs) (Yağci et al. 2008).

Treatment modality was not associated with the survival time in our patient population, although a tendency towards longer survival time for patients receiving localised (surgery or radiation therapy) or systemic treatment (vs. no treatment) was found. Long term local control of the tumour can be achieved in patients with histiocytic sarcoma (Skorupski et al. 2009; Marconato et al. 2020). In a recent study, no significant differences in the local tumour control were found in patients treated with various protocols of radiation therapy or surgery in patients with periarticular histiocytic sarcoma (Marconato et al. 2020). In our study patients were treated

with different protocols and systemic treatment was not administered consistently to the entire population. Therefore, it is plausible that this, in combination with the low sample size reduced the power of the study to detect significant differences between the treatment groups.

This study presented several limitations, mainly due to its retrospective nature.

As previously mentioned, there was no standardisation of the staging and diagnosis, which may have caused an underestimation of the number of patients categorised as having disseminated disease. In addition, the diagnosis of histiocytic sarcoma was made by cytology alone in some cases which could have resulted in misdiagnosis in some cases, although the diagnosis would have been based on a combination of cytological findings and other clinical features suggestive of histiocytic sarcoma (e.g. tumour site, breed). Although histopathology and immunohistochemistry are considered the gold standard for the diagnosis of histiocytic neoplasia, previous literature suggests that the distinction of histiocytic sarcoma from other neoplastic diseases by cytology alone is feasible (Cannon et al. 2015; Brown et al. 1994; Erich et al. 2018; Wiley et al. 2009). Hence, cytology alone can be used if a biopsy would cause an increase risk

of complications or morbidity (Cannon et al. 2015; Wiley et al. 2009). Lastly, some of the previous major retrospective studies used cytology as the sole diagnosis in a percentage of cases, reflecting the reality of the clinical presentation and diagnosis of the disease (Cannon et al. 2015; Skorupski et al. 2009; Marlowe et al. 2018). Another limitation is the lack of standardisation of the treatment. This was particularly evident in patients treated with radiation therapy that received different dose intensities and number of fractions and in the choice of rescue protocol that have been used. However, radiation therapy in the present study was mainly performed in a palliative setting, and it is unlikely that the use of different protocols has majorly affected the overall survival time. Serum CRP concentrations were also measured using stored, frozen serum samples for a minority of the cases (6/35 cases), which may have further confounded the results of the study, and although there is no data regarding the long term stability of canine CRP at -80°C , one study in humans only documented a small, but significant, increase (0.34 mg/L) in serum/plasma CRP concentrations after 13 years of storage (postulated to be secondary to sample dehydration) (Ishikawa et al. 2007). Therefore we believe it is unlikely that serum CRP concentrations obtained from frozen samples would have been significantly elevated at the time of measurement compared to the time of sampling. A last limitation of the study is the small sample size. This could have caused a reduction in the statistical validity of some analysis, particularly the association between treatment and survival time, and the association between tumour stage and serum CRP concentrations. For this reason, larger studies with a standardisation of the staging system will be required.

Conclusions

To the authors' knowledge, this is the first study describing serum CRP concentrations as a prognostic marker in different breeds of dogs with histiocytic sarcoma, and it provides proof of concept that serum CRP concentrations are associated with survival time in these patients. Decreasing age was also independently associated with reduced survival in our study. Even if serum CRP was associated with prognosis, higher serum concentration of the protein were not associated with a more advanced stage of the disease or with the primary localisation of the tumour. The association of serum CRP concentrations, tumour stage and age with prognosis in histiocytic sarcoma should be evaluated in larger studies that also evaluate other established prognostic factors.

Acknowledgements Preliminary data were presented as an abstract at the BSAVA Virtual Congress 2021.

Funding This research received no external funding.

Compliance with ethical standards

Ethical approval For this type of study formal consent is not required.

Informed consent statement For this type of study informed consent is not required.

Consent for publication For this type of study consent for publication is not required.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship or publication of this article. All authors have read and agreed to the published version of the manuscript.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

- Allin KH, Bojesen SE, Nordestgaard BG (2009) Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. *J Clin Oncol* 27:2217–2224. <https://doi.org/10.1200/JCO.2008.19.8440>
- Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow? *Lancet* 357:539–545. [https://doi.org/10.1016/S0140-6736\(00\)04046-0](https://doi.org/10.1016/S0140-6736(00)04046-0)
- Brown DE, Thrall MA, Getzy DM, Weiser MG, Ogilvie GK (1994) Cytology of canine malignant histiocytosis. *Vet Clin Pathol* 23:118–123. <https://doi.org/10.1111/j.1939-165x.1994.tb00921.x>
- Cannon C, Borgatti A, Henson M, Husbands B (2015) Evaluation of a combination chemotherapy protocol including lomustine and doxorubicin in canine histiocytic sarcoma. *J Small Anim Pract* 56:425–429. <https://doi.org/10.1111/jsap.12354>
- Ceron JJ, Eckersall PD, Martı́nez-Subiela S (2005) Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Vet Clin Pathol* 34:85–99. <https://doi.org/10.1111/j.1939-165x.2005.tb00019.x>
- Constantino-Casas F, Mayhew D, Hoather TM, Dobson JM (2011) The clinical presentation and histopathologic-immunohistochemical classification of histiocytic sarcomas in the Flat Coated Retriever. *Vet Pathol* 48:764–771. <https://doi.org/10.1177/0300985810385153>
- Dervisis NG, Kiupel M, Qin Q, Cesario L (2017) Clinical prognostic factors in canine histiocytic sarcoma. *Vet Comp Oncol* 15:1171–1180. <https://doi.org/10.1111/vco.12252>

- Dethlefsen C, Højfeldt G, Hojman P (2013) The role of intratumoral and systemic IL-6 in breast cancer. *Breast Cancer Res Treat* 138:657–664. <https://doi.org/10.1007/s10549-013-2488-z>
- Dobson JM (2013) Breed-predispositions to cancer in pedigree dogs. *ISRN Vet Sci* 2013:941275. <https://doi.org/10.1155/2013/941275>
- Erich SA, Constantino-Casas F, Dobson JM, Teske E (2018) Morphological distinction of histiocytic sarcoma from other tumor types in Bernese mountain dogs and flatcoated retrievers. *In Vivo* 32:7–17. <https://doi.org/10.21873/invivo.11198>
- Fidel J, Schiller I, Hauser B, Jausi Y, Rohrer-Bley C, Roos M, Kaser-Hotz B (2006) Histiocytic sarcomas in flat-coated retrievers: a summary of 37 cases (November 1998–March 2005). *Vet Comp Oncol* 4:63–74. <https://doi.org/10.1111/j.1476-5810.2006.00090.x>
- Fulmer AK, Mauldin GE (2007) Canine histiocytic neoplasia: an overview. *Can Vet J* 48(1041–1043):1046–1050
- Ishikawa, S.; Kayaba, K.; Gotoh, T.; Nakamura, Y.; Kario, K.; Ito, Y.; Kajii, E.; Cohort, J. Comparison of C-reactive protein levels between serum and plasma samples on long-term frozen storage after a 13.8 year interval: the JMS Cohort Study. *J Epidemiol* 2007, 17, 120–124, doi: 10.2188/jea.17.120.
- Kezer KA, Barber LG, Jennings SH (2018) Efficacy of dacarbazine as a rescue agent for histiocytic sarcoma in dogs. *Vet Comp Oncol* 16:77–80. <https://doi.org/10.1111/vco.12314>
- Klahn SL, Kitchell BE, Dervisis NG (2011) Evaluation and comparison of outcomes in dogs with periarticular and nonperiarticular histiocytic sarcoma. *J Am Vet Med Assoc* 239:90–96. <https://doi.org/10.2460/javma.239.1.90>
- Klenner S, Bauer N, Moritz A (2010) Evaluation of three automated human immunoturbidimetric assays for the detection of C-reactive protein in dogs. *J Vet Diagn Invest* 22:544–552. <https://doi.org/10.1177/104063871002200408>
- Kuntz CA, Dernel WS, Powers BE, Devitt C, Straw RC, Withrow SJ (1997) Prognostic factors for surgical treatment of soft-tissue sarcomas in dogs: 75 cases (1986–1996). *J Am Vet Med Assoc* 211:1147–1151
- Lenz JA, Furrow E, Craig LE, Cannon CM (2017) Histiocytic sarcoma in 14 miniature schnauzers - a new breed predisposition? *J Small Anim Pract* 58:461–467. <https://doi.org/10.1111/jsap.12688>
- Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. *Nature* 454:436–444. <https://doi.org/10.1038/nature07205>
- Marconato L, Sabattini S, Buchholz J, Polton G, Finotello R, Martano M, Willman M, Massari F, Agnoli C, Gedon J et al (2020) Outcome comparison between radiation therapy and surgery as primary treatment for dogs with periarticular histiocytic sarcoma: an Italian Society of Veterinary Oncology study. *Vet Comp Oncol* 18:778–786. <https://doi.org/10.1111/vco.12609>
- Marlowe KW, Robat CS, Clarke DM, Taylor A, Touret M, Husbands BD, Vail DM (2018) Primary pulmonary histiocytic sarcoma in dogs: A retrospective analysis of 37 cases (2000–2015). *Vet Comp Oncol* 16:658–663. <https://doi.org/10.1111/vco.12437>
- Merlo A, Rezende BC, Franchini ML, Simões DM, Lucas SR (2007) Serum C-reactive protein concentrations in dogs with multicentric lymphoma undergoing chemotherapy. *J Am Vet Med Assoc* 230:522–526. <https://doi.org/10.2460/javma.230.4.522>
- Moore PF, Affolter VK, Vernau W (2006) Canine hemophagocytic histiocytic sarcoma: a proliferative disorder of CD11d+ macrophages. *Vet Pathol* 43:632–645. <https://doi.org/10.1354/vp.43-5-632>
- Mukorera V, Dvir E, van der Merwe LL, Goddard A (2011) Serum C-reactive protein concentration in benign and malignant canine spirocercosis. *J Vet Intern Med* 25:963–966. <https://doi.org/10.1111/j.1939-1676.2011.0733.x>
- Murata H, Shimada N, Yoshioka M (2004) Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet J* 168:28–40. [https://doi.org/10.1016/S1090-0233\(03\)00119-9](https://doi.org/10.1016/S1090-0233(03)00119-9)
- Nakamura M, Takahashi M, Ohno K, Koshino A, Nakashima K, Setoguchi A, Fujino Y, Tsujimoto H (2008) C-reactive protein concentration in dogs with various diseases. *J Vet Med Sci* 70:127–131. <https://doi.org/10.1292/jvms.70.127>
- Nielsen L, Toft N, Eckersall PD, Mellor DJ, Morris JS (2007) Serum C-reactive protein concentration as an indicator of remission status in dogs with multicentric lymphoma. *J Vet Intern Med* 21:1231–1236. <https://doi.org/10.1892/07-058.1>
- Nikolic Nielsen L, Kjelgaard-Hansen M, Kristensen AT (2013) Monocyte chemotactic protein-1 and other inflammatory parameters in Bernese Mountain dogs with disseminated histiocytic sarcoma. *Vet J* 198:424–428. <https://doi.org/10.1016/j.tvjl.2013.07.030>
- Nivy R, Caldin M, Lavy E, Shaabon K, Segev G, Aroch I (2014) Serum acute phase protein concentrations in dogs with spirocercosis and their association with esophageal neoplasia - a prospective cohort study. *Vet Parasitol* 203:153–159. <https://doi.org/10.1016/j.vetpar.2014.02.001>
- Planellas M, Bassols A, Siracusa C, Saco Y, Giménez M, Pato R, Pastor J (2009) Evaluation of serum haptoglobin and C-reactive protein in dogs with mammary tumors. *Vet Clin Pathol* 38:348–352. <https://doi.org/10.1111/j.1939-165X.2009.00139.x>
- Pradel J, Berlatto D, Dobromylskyj M, Rasotto R (2018) Prognostic significance of histopathology in canine anal sac gland adenocarcinomas: Preliminary results in a retrospective study of 39 cases. *Vet Comp Oncol* 16:518–528. <https://doi.org/10.1111/vco.12410>
- Purzycka K, Peters LM, Elliott J, Lamb CR, Priestnall SL, Hardas A, Johnston CA, Rodriguez-Piza I (2020) Histiocytic sarcoma in miniature schnauzers: 30 cases. *J Small Anim Pract* 61:338–345. <https://doi.org/10.1111/jsap.13139>
- Raposo TP, Beirão BC, Pang LY, Queiroga FL, Argyle DJ (2015) Inflammation and cancer: till death tears them apart. *Vet J* 205:161–174. <https://doi.org/10.1016/j.tvjl.2015.04.015>
- Saellström S, Sharif H, Jagarlamudi KK, Rönnerberg H, Wang L, Eriksson S (2022) Serum TK1 protein and C-reactive protein correlate to treatment response and predict survival in dogs with hematologic malignancies. *Res Vet Sci* 145:213–221. <https://doi.org/10.1016/j.rvsc.2022.02.019>
- Satter EK, High WA (2008) Langerhans cell histiocytosis: a review of the current recommendations of the Histiocyte Society. *Pediatr Dermatol* 25:291–295. <https://doi.org/10.1111/j.1525-1470.2008.00669.x>
- Selting KA, Sharp CR, Ringold R, Knouse J (2015) Serum thymidine kinase 1 and C-reactive protein as biomarkers for screening clinically healthy dogs for occult disease. *Vet Comp Oncol* 13:373–384. <https://doi.org/10.1111/vco.12052>
- Selting KA, Ringold R, Husbands B, Pithua PO (2016) Thymidine Kinase Type 1 and C-reactive protein concentrations in dogs with spontaneously occurring cancer. *J Vet Intern Med* 30:1159–1166. <https://doi.org/10.1111/jvim.13954>
- Skorupski KA, Clifford CA, Paoloni MC, Lara-Garcia A, Barber L, Kent MS, LeBlanc AK, Sabhlok A, Mauldin EA, Shofer FS et al (2007) CCNU for the treatment of dogs with histiocytic sarcoma. *J Vet Intern Med* 21:121–126. [https://doi.org/10.1892/0891-6640\(2007\)21\[121:cfttd\]2.0.co;2](https://doi.org/10.1892/0891-6640(2007)21[121:cfttd]2.0.co;2)
- Skorupski KA, Rodriguez CO, Krick EL, Clifford CA, Ward R, Kent MS (2009) Long-term survival in dogs with localized histiocytic sarcoma treated with CCNU as an adjuvant to local therapy. *Vet Comp Oncol* 7:139–144. <https://doi.org/10.1111/j.1476-5829.2009.00186.x>
- Sylman JL, Mitrugno A, Atallah M, Tormoen GW, Shatzel JJ, Tassi Yunga S, Wagner TH, Leppert JT, Mallick P, McCarty OJT (2018) The predictive value of inflammation-related peripheral blood measurements in cancer staging and prognosis. *Front Oncol* 8:78. <https://doi.org/10.3389/fonc.2018.00078>

- Tecles F, Caldín M, Zanella A, Membiela F, Tvarijonaviciute A, Subiela SM, Cerón JJ (2009) Serum acute phase protein concentrations in female dogs with mammary tumors. *J Vet Diagn Invest* 21:214–219. <https://doi.org/10.1177/104063870902100206>
- Wiley J, Walton R, Kennedy D (2009) Comparison of cytology, flow cytometry using CD18 and histopathology with immunohistochemistry (CD18) for the diagnosis of canine histiocytic sarcoma. Proceedings of the Veterinary Cancer Society. Austin, TX, USA, October, 15, 28.
- Yağci B, Varan A, Çağlar M, Söylemezoğlu F, Sungur A, Orhan D, Yalçın B, Akyüz C, Kutluk T, Büyükpamukçu M (2008) Langerhans cell histiocytosis: retrospective analysis of 217 cases in a single center. *Pediatr Hematol Oncol* 25:399–408. <https://doi.org/10.1080/08880010802107356>
- Yamashita K, Fujinaga T, Miyamoto T, Hagio M, Izumisawa Y, Kotani T (1994) Canine acute phase response: relationship between serum cytokine activity and acute phase protein in dogs. *J Vet Med Sci* 56:487–492. <https://doi.org/10.1292/jvms.56.487>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.