ORIGINAL ARTICLE

Biochemical and haemostatic variables associated with metastasis in dogs with carcinoma or sarcoma

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Objectives: Identify alterations in biochemical and haemostatic variables between tumour-bearing dogs with and without metastasis that can be used to predict the presence of metastasis.

Materials and Methods: Thirty dogs with sarcoma and 29 with carcinoma were included in the analysis. Serum biochemistry profiles and haemostatic variables (haematocrit value, platelet count, thromboelastography (TEG), fibrinogen, Factor X, VII, antithrombin activity and D-dimer concentration) were measured for all dogs. All dogs underwent complete post-mortem and histopathological evaluations. For tumour-bearing dogs without intracavitary haemorrhage, measured variables were compared between dogs with and without metastasis, and univariate and multivariable analysis were performed to identify predictors of metastasis.

Results: Metastasis was identified in 31 of 59 (53%) dogs, of which 5 of 31 (16%) had metastasis to the regional lymph node only and 26 of 31 (84%) had distant metastasis. Sodium, ionised calcium, TEG lysis % at 30 and 60 minutes (Ly60) were significantly lower in tumour-bearing dogs without intracavitary haemorrhage with metastasis compared to dogs without metastasis. Multivariable analysis identified sodium <142.5 mmol/L as 64% sensitive ($CI_{95\%}$:45% to 82%) and 63% specific ($CI_{95\%}$:44% to 81%); and Ly60 < 1.0% as 68% sensitive ($CI_{95\%}$: 49% to 88%) and 78% specific ($CI_{95\%}$:61% to 95%) for prediction of the presence of metastasis. Parallel interpretation of lower sodium and decreased Ly60 resulted in high sensitivity (96%) for the presence of metastasis.

Clinical Significance: Sodium and TEG-based decreased fibrinolysis were associated with metastasis in tumour-bearing dogs without haemorrhage; when identified, they should prompt further diagnostics to detect possible metastasis of a primary carcinoma or sarcoma.

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INTRODUCTION

In people with cancer, metastasis is the primary cause of morbidity and is responsible for approximately 90% of mortalities (Chaffer & Weinberg, 2011). In dogs, the incidence of metastasis of carcinoma and sarcoma is dependent on the primary tumour type and ranges between 5% and 88% (Miles et al., 1990; Waters et al., 1988). There are few veterinary

studies that have investigated routinely used biochemical and haemostatic variables as predictors of metastasis in dogs with carcinoma or sarcoma.

The pathogenesis of metastasis is complex and involves cell migration, intravasation into vasculature or lymphatics, survival in circulation, extravasation and colonisation at the new tumour site (Majidpoor & Mortezaee, 2021). The clinical identification of metastasis is through a combination of diagnostic procedures, including two- and three-dimensional imaging, cytology and biopsies (Greene, 2020). These diagnostic modalities usually require expensive equipment and specialized expertise for interpretation, often making the clinical diagnosis of metastasis challenging. Standard laboratory biochemistry and haemostatic profiles are considered routine in the workup of cancer patients. If these profiles could aid in the early prediction of the presence of metastasis, the expense, time and expertise involved in the identification of metastasis would be significantly reduced. This could also result in screening and selection of patients for more advanced diagnostics for metastasis detection and potentially earlier intervention, targeted therapy, reduced recurrence rates and longer survival times.

In people, clinico-pathological changes associated with metastasis in various cancer types include anaemia (Lin et al., 2019), neutrophilia (Kim et al., 2022; Su et al., 2015; Tavares-Murta et al., 2010), thrombocytosis (Kocak et al., 2014; Lee et al., 2015; Maraz et al., 2013), hypercalcaemia (Coleman & Rubens, 1987) and hyponatraemia (Castillo et al., 2016). In addition, haemostatic abnormalities are common, with the relationship between coagulopathy and metastasis well recognised but poorly understood (Walsh et al., 2019). Malignancy-associated coagulopathy encompasses a spectrum of haemostatic derangements, platelet dysfunction and vascular disturbances typically characterised by a hypercoagulable state with hypofibrinolysis (Walsh et al., 2019). Approximately 10% to 20% of people with cancer present with concomitant thromboembolic disease, which is associated with higher clinical staging (Guijarro et al., 2016; Horsted et al., 2012; Saeger & Genzkow, 1994; Sorensen et al., 2000; Timp et al., 2013). Activation of the coagulation cascade and haemostatic dysfunction can enhance metastatic efficiency through various mechanisms including formation of fibrin–platelet–tumour cell complexes, with microthrombi playing an integral role in metastasis (Camerer et al., 2004; Francis et al., 1998; Palumbo et al., 2002).

In dogs, clinico-pathological changes reported to be associated with metastasis include neutrophilic leukocytosis (Ziccardi et al., 2022), higher total protein (Duda et al., 2017) and hypercalcaemia (Coady et al., 2019). Haemostatic changes associated with metastasis in dogs with various tumours include thrombocytopenia, hyperfibrinogenaemia, prolonged activated partial thromboplastin time, hyper- or hypocoagulability based on thromboelastography (TEG), increased D-dimer concentration and decreased antithrombin (AT) activity (Andreasen et al., 2012; Kristensen et al., 2008; Masyr et al., 2021; Stockhaus et al., 1999). Haemostatic dysfunction consistent with non-overt and overt disseminated intravascular coagulation (DIC) was previously reported in dogs with carcinoma or sarcoma and was postulated to contribute to microthrombi formation (Pazzi et al., 2023). Additionally, the presence of intracavitary haemorrhagic effusions or overt DIC has a significant effect on haematological and haemostatic variables, including anaemia, thrombocytopenia, reduced fibrinogen and clotting times and increased D-dimers (Dewhurst et al., 2008; Fletcher et al., 2016; Hammer et al., 1991; Kristensen et al., 2008; Nelson & Andreasen, 2003; Pazzi et al., 2023).

The aims of this study were to (1) identify differences in the biochemical and haemostatic variables between tumour-bearing dogs with carcinoma or sarcoma, with and without metastasis and identify if any of the variables can predict the presence of metastasis; and (2) evaluate the effect of intra-cavitary haemorrhage on the differences identified between tumour-bearing dogs with and without metastasis. We hypothesised that haemostatic changes in tumour-bearing dogs with metastasis would be consistent with malignancy-associated coagulopathy, similar to that reported in people. The effect of intracavitary haemorrhage would predominantly be reflected by changes in haemostatic variables consistent with blood loss. Lastly, one or more of the biochemical or haemostatic variables would be a reasonable predictor of the presence of metastasis.

MATERIALS AND METHODS

Study animals

The prospective cross-sectional study design, as previously described (Pazzi et al., 2023), is briefly outlined. Client-owned dogs presenting for veterinary care to the Onderstepoort Veterinary Academic Hospital from December 2018 to September 2000 were prospectively included in this study. Research ethics approval was granted by the Research Ethics Committee of the University of Pretoria. Informed owner consent was obtained for all dogs included in this study.

Dogs above 1 year of age, weighing greater than 5 kilograms, diagnosed clinically and cytologically with carcinoma or sarcoma and without clinical evidence of concomitant disease were eligible for inclusion. Dogs were not considered for inclusion if they had evidence of any unrelated inflammatory condition identified on clinical examination; treatment with any medication in the preceding 2 weeks before presentation (with the exception of corticosteroids and non-steroidal anti-inflammatory drugs); or second tumour type identified on clinical examination. Those that were to be euthanased due to a poor prognosis or financial constraints pertaining to their primary tumour, defined as the reason for presentation and subsequent euthanasia, were prospectively enrolled as the study population. The presence and subsequent classification of a primary sarcoma or carcinoma was confirmed based on histopathology of biopsy samples taken of the primary tumour during post-mortem examination.

Dogs were excluded if visible macrothrombi, confirmed by histopathology, were found in any blood vessels during post-mortem examination because the development of macrothrombi is expected to cause haemostatic changes including hypercoagulability, decreased AT and increased D-dimers that could

influence how subsequent group comparisons were interpreted (Bauer & Moritz, 2013; Dengate et al., 2016; Katsumura & Ohtsubo, 1995; Lippi et al., 2010; Pazzi et al., 2022). Dogs were not excluded if inflammation was identified on post-mortem examination or histopathology.

Sample collection and diagnostic testing

Blood samples were collected in a standard order, with minimal tissue factor contamination, using vacuum assistance, as previously described (Pazzi et al., 2023). Blood was collected from all tumour-bearing dogs less than 40 minutes prior to euthanasia. Haematocrit value and platelet count are variables affecting TEG (Bochsen et al., 2011; Bowbrick et al., 2003), and were determined using the EDTA sample on the Advia 2120i (Siemens, Berlin Germany) automated haematology analyser within 2 h of collection.

Serum, separated by centrifugation, was stored at −80°C, and biochemical analysis was performed as a batch on the Cobas Integra 400 plus analyser (Roche, Basel, Switzerland). Serum biochemistry assays included total serum protein, albumin, globulin, alanine aminotransferase, alkaline phosphatase, urea, creatinine and previously validated for dogs—C-reactive protein (Gentian, Moss Norway) (Covin et al., 2021; Hillstrom et al., 2014) and serum amyloid A (VET SAA, Eiken, Japan) (Christensen et al., 2012, 2013). Sodium, potassium and ionised calcium (iCa) were determined using the Rapidpoint 500 blood gas analyzer (Siemens, Berlin, Germany).

Haemostatic variables were evaluated using the sodium citrate sample. Thromboelastography (TEG® 5000 Thrombelastograph® Haemostasis System; Haemonetics Corporation, Braintree, Massachusetts, United States), using kaolin as an activator, was performed on whole blood 30 minutes after blood sample collection, according to manufacturer instructions. Variables derived from the thromboelastogram included reaction time from the start of the tracing to the point where the lines diverge 2 mm (R), time from initial clot formation to reach 20 mm clot strength (K), rapidity of fibrin build-up and cross-linking (α-angle), maximum clot strength or amplitude (MA), calculated measure of the overall clot strength (G), lysis at 30 minutes (Ly30) and lysis at 60 minutes (Ly60) and were interpreted as previously described (Pazzi et al., 2023). Following centrifugation, the citrated plasma was removed and stored at −80°C for batch analysis of AT activity (Precimat Chromogen, Roche, Basel, Switzerland) on the Cobas Integra 400 plus analyzer (Roche, Basel, Switzerland); D-dimer concentration was determined using a quantitative turbidimetric immunoassay, calibrated with the manufacturer's human D-dimer standard (HemosIL, Ilex, South Africa). D-dimer and fibrinogen concentrations, and Factors VII and X activity were measured (HemosIL, Ilex, South Africa), using the ACL Elite® Analyzer (Instrumentation laboratories, Massachusetts, United States). For AT activity, the patient activity was normalised against the AT activity of a pooled control sample, consisting of 10 healthy dogs, which was presumed to have a 100% activity. For Factors VII and X activity, the coagulation factor activity of the tumour-bearing dogs was normalised against the mean activity of 20 healthy age-controlled dogs' Factors VII and X taken from a previous study (Pazzi et al., 2023), presumed to have a 100% activity.

Post-mortem examination

A full post-mortem examination was performed by the first author on all tumour-bearing dogs enrolled in the study, within 2 h of euthanasia. Intracavitary heammorhage was defined as free fluid within the peritoneal, pleural or pericardial space and with a haematocrit value similar to the patients. Samples of the primary tumour, regional lymph node (when identified) and other additional specific organ sampling sites were placed in formalin (10%) for routine haematoxylin and eosin staining and light microscopic examination. Additional specific organ sampling included multiple 1 cm^3 sections of all the parenchymatous organs—details in File S1. In addition, each parenchymatous organ was routinely cut sagittally and transversally into approximately 1 cm blocks to visually evaluate and physically palpate for macroscopic abnormalities. All macroscopically abnormal areas or areas suspicious of metastasis were sampled for histopathological evaluation. Histopathological evaluation was performed by a board-certified pathologist. Metastasis was defined as the presence of macro or microscopic evidence, confirmed on histopathology, of the same tumour cells from the primary tumour in a distant tissue, organ or lymph node. Microthrombi were identified histologically, as previously defined (Pazzi et al., 2022). Additional observations recorded included: tumour location; presence of macroscopic tumour ulceration or histological evidence of inflammation or necrosis of the tumour or another organ; secondary or tertiary primary tumours identified during post-mortem or histopathology.

Statistical analysis

Data were analysed using the SPSS 27 software (IBM SPSS Inc. Armonk, NY). All measured variables were tested for a normal distribution using a Shapiro-Wilks test and evaluation of histograms. Data that were right-skewed were natural log transformed prior to statistical analysis. If transformation did not improve the distributional form, then nonparametric statistical methods were employed.

Prevalence of metastasis was estimated using total dogs with metastasis of the primary tumour divided by the total number of tumour-bearing dogs. The prevalence of metastasis was determined for the carcinoma and sarcoma groups independently. Tumour-bearing dogs with metastasis of the primary tumour were compared to dogs without metastasis, regardless of primary tumour type, after exclusion of dogs with intracavitary haemorrhagic effusions. To determine the effect of intracavitary haemorrhage on differences identified between tumour-bearing dogs with and without metastasis, dogs with intracavitary haemorrhage were then included with other tumour-bearing dogs and the analysis was repeated. The institutional laboratory reference intervals are included in the results tables for reference.

Normally distributed data were compared using Student's *t*-tests. Non-normally distributed data were compared among groups using Mann–Whitney *U*-tests. The association between measured variables and predictors of the presence of metastasis in tumour-bearing dogs without intracavitary haemorrhage was further evaluated using binary logistic regression. Univariate

screening models for the predictive association with metastatic disease were fit for the medians of each continuous variable for tumour-bearing dogs, and variables with P values < 0.2 were selected for multivariable modelling. Collinearity was assessed for all variables identified during the univariate screening procedures and prior to fitting multivariable models. Substantial collinearity was defined as a variance inflation factor (VIF) > 5. Collinear variables were excluded, and VIF reassessed until all remaining variables for multivariable modelling were VIF < 5. Multivariable models were fit starting with all non-collinear variables identified during the univariate screening process, with variables subsequently removed one-by-one based on the largest Wald P value until all remaining variables were P < 0.05.

Post hoc analysis was performed using receiver-operating curves to determine the sensitivity and specificity of significant predictors for the detection of metastasis identified using the multivariable model. Optimal cut-off values were determined based on the Youden index (sensitivity + specificity − 1), rather than median values used in logistical regression and in-series and in-parallel interpretations were employed to evaluate the accuracy of using multiple tests in combination. Unless stated otherwise, significance was set as P < 0.05.

RESULTS

Study population characteristics

Sixty-two tumour-bearing dogs were enrolled. Three dogs with macrothrombi were subsequently excluded. The remaining tumour-bearing group consisted of 59 dogs and 22 breeds (Table S1).

Descriptive statistics in dogs with carcinoma or sarcoma

Thirty primary sarcomas and 29 primary carcinomas were identified (Table 1, Fig. 1). Regional lymph nodes were identified and sampled in 46 of 59 (78%) dogs. Metastasis to various sites was identified in 31 of 59 (53%) dogs, of which 5 of 31 (16%) had detectable metastasis to the regional lymph node only, 7 of 31 (23%) metastasis to the regional lymph node and distant metastasis and 19 of 31 (61%) only distant metastasis (Table 1, Fig. 1). Metastasis was identified in 15 of 29 (52%) dogs with carcinoma and 16 of 30 (53%) dogs with sarcoma. Of the dogs with carcinoma, 4 of 15 (27%) had metastasis to the regional lymph node only, 6 of 15 (40%) metastasis to the regional lymph node and distant metastasis and 5 of 15 (33%) only distant metastasis. Of the dogs with sarcoma, 1 of 16 (6%) had metastasis to the regional lymph node only, 1 of 16 (6%) had metastasis to the regional lymph node and distant metastasis and 14 of 16 (88%) had only distant metastasis. Therefore, of the dogs with metastasis, lymph node metastasis was present in 12 of 31 (39%) dogs, and was identified in 10 of 29 (34%) dogs with carcinoma and 2 of 30 (7%) dogs with sarcoma. Distant metastasis, present in 26 of 59 (44%) dogs, was identified in 11 of 29 (38%) dogs with carcinoma and 15 of 30 (50%) dogs with sarcoma. Secondary primary tumours were identified in 10 of 29 (34%) dogs with carcinoma and 14 of 30 (47%) dogs with sarcoma (Table S2). Evidence of metastasis of the secondary primary tumour was identified in two dogs, both with primary carcinomas, and both with metastatic melanomas. One of these dogs also had metastasis of the primary tumour while the other did not.

Intracavitary haemorrhage was identified in 6 of 31 (19%) dogs with metastasis, and in 4 of 28 (14%) of the dogs without metastasis. Of the 31 dogs with metastatic disease, 16 (52%) had concurrent microthrombi, of which 9 (56%) were intra-tumoural only, 4 (25%) were both intra-tumoural and distant and 3 (19%) were distant only. Macroscopic ulceration or histological evidence of inflammation or necrosis of the tumour or another organ was identified in 27 of 31 (87%) dogs with metastasis and 21 of 28 (75%) dogs without metastasis (Table S2). Concurrent administration of corticosteroids was identified in 3 of 59 (5%) dogs and non-steroidal anti-inflammatory drugs in 5 of 59 (8%) dogs, as previously described (Pazzi et al., 2023).

Comparison of haematological, biochemical and haemostatic variables between tumour-bearing dogs with and without metastasis

After exclusion of dogs with intracavitary haemorrhage, variables were compared in 49 dogs; 25 with metastasis and 24 without metastasis. The sodium and iCa concentrations were significantly lower in tumour-bearing dogs with metastatic disease compared to tumour-bearing dogs without metastasis (P = 0.017 and P = 0.002, respectively; Table 2, Fig. 2).

Ly30 and Ly60 were significantly lower in dogs with metastasis compared to those without metastasis (P = 0.007 and P = 0.004 respectively, Fig. 3). No other differences in TEG or haemostatic variables were identified between tumour-bearing dogs with or without metastatic disease.

When dogs with intracavitary haemorrhage were included in the statistical analysis, tumour-bearing dogs with (n = 31) and without metastasis (n = 28) were compared. All significant findings remained (Table 3). In addition, D-dimer concentrations were significantly higher (P = 0.026), and factor X and AT activities were significantly lower in dogs with metastasis compared to those without (P = 0.023 and P = 0.036, respectively).

Variables associated with metastatic disease

Variables that had a P value < 0.2 in the univariate prediction of metastatic disease were total serum protein, sodium, iCa, Ly30, Ly60, Factor VII, Factor X and AT activities (Table S3). The final multivariable model identified sodium (odds ratio (OR): 0.182, $CI_{95\%}$: 0.046 to 0.719; P = 0.015) and Ly60 (OR 0.219, $CI_{95\%}$: 0.055 to 0.867; P = 0.030) as significant predictors for the detection of metastasis. The median cut-off values and respective sensitivity and specificity for each variable for prediction of metastasis were <142.5 mmol/L, 64% and 63% for sodium (P = 0.010) (Table 4); <1.0%, 68% and 78% for Ly60 (P = 0.001). Serial interpretation of hyponatraemia (<141.6 mmol/L) and decreased lysis (Ly 60 < 2.1%) resulted in high specificity and low sensitivity for the prediction of metastasis, while parallel interpretation of these two variables resulted in high sensitivity but low specificity.

Table 1. Primary tumours of dogs with carcinoma or sarcoma and site(s) of metastasis of the primary tumour

Primary tumour	Metastatic site of primary tumour
Sarcoma (30)	
Subcutaneous soft tissue sarcoma	Lung
Subcutaneous soft tissue sarcoma	N/A
Subcutaneous soft tissue sarcoma	N/A
Splenic haemangiosarcoma	Right atrium, liver
Splenic haemangiosarcoma†	Lymph node, liver kidney, omentum
Splenic haemangiosarcoma†	N/A
Right atrial haemangiosarcoma†	Spleen, lungs
Right atrial haemangiosarcoma†	N/A
Splenic and right atrial haemangiosarcoma†	Liver, omentum
Splenic and right atrial haemangiosarcoma†	N/A
Hepatic haemangiosarcoma†	Omentum
Hepatic haemangiosarcoma†	Diaphragm, lungs
Cutaneous haemangiosarcoma	N/A
Cutaneous haemangiosarcoma	N/A
Subcutaneous haemangiosarcoma	Lungs
Muscle haemangiosarcoma	Liver, right atrium, lungs
Mammary osteosarcoma	Lymph node
Mammary osteosarcoma	Lungs
Oesophageal osteosarcoma	Aorta, liver, lungs
Oesophageal osteosarcoma	Kidney, liver, lung, caudal vena cava
Rib chondroblastic osteosarcoma	Lung
Maxillary osteosarcoma	N/A
Mandibular osteosarcoma	N/A
Mixed peri-orbital anaplastic sarcoma	Liver, pancreas, omentum, cardiac muscle, dermis/subcutis and muscle
Splenic stromal sarcoma	Liver
Muscle soft tissue sarcoma	N/A
Mixed mammary sarcoma	N/A
Hepatic spindle cell sarcoma†	N/A
Cutaneous soft tissue sarcoma	N/A
Mandibular soft tissue sarcoma	N/A
Carcinoma (29)	
Complex mammary carcinoma	Lymph node
Complex mammary carcinoma	Lymph node
Complex mammary carcinoma	N/A
Complex mammary carcinoma	N/A
Simple tubular mammary carcinoma	Lymph node
Simple tubular mammary carcinoma	Lungs
Simple tubular mammary carcinoma	N/A
Mixed mammary carcinoma	Lymph node
Mixed mammary carcinoma	Lymph node, lungs
Anaplastic mammary carcinoma	Lymph node, lungs
Simple solid mammary carcinoma	N/A
Simple tubulopapillary mammary carcinoma	N/A
Mammary ductal carcinoma	N/A
Mixed type carcinoma and simple tubulopapillary mammary carcinoma	N/A
Spindle cell mammary carcinoma	N/A
Cutaneous squamous cell carcinoma	N/A
Cutaneous squamous cell carcinoma	N/A
Hepatocellular carcinoma†	Liver
Hepatocellular carcinoma	N/A
Apocrine gland adenocarcinoma (anal sac)	Lymph node, spleen, adrenal gland, lungs
Carcinoma (papillary and solid)—source unknown	Lungs
Tubular mesothelioma	Lungs
Anaplastic cholangiocellular carcinoma	Lymph node, liver, peri-pancreatic connective tissue, bronchial lnn., lungs
Thyroid microfollicular carcinoma	Rib
Urothelial carcinoma	Lymph node, rib, gastric and tracheobronchial lymph node
Pulmonary carcinomatosis	Lymph node, Adrenal gland, liver, kidney
Pulmonary carcinoma (tubulopapillary type)	N/A
Sinonasal transitional carcinoma	N/A
Solid scirrhous prostatic carcinoma	N/A

†Indicates intracavitary haemorrhage at post-mortem
N/A not applicable

DISCUSSION

In this study, dogs diagnosed with carcinoma or sarcoma, with metastasis and no intracavitary haemorrhage, had significantly lower sodium, iCa, TEG Ly30 and Ly60 than dogs without metastasis. Lower sodium and decreased fibrinolysis, based on Ly60, were associated with metastasis, with moderate sensitivity and specificity. Serial interpretation of hyponatraemia and decreased fibrinolysis results in high specificity and low sensitivity for the association with metastasis, while the inverse was observed with parallel interpretation of these two variables.

Sodium and iCa were the only biochemistry variables significantly different in tumour-bearing dogs with metastasis compared to tumour-bearing dogs without metastasis. Hyponatraemia is reported in 25% to 76% of people with cancer and is a negative prognostic indicator associated with increased mortality and morbidity (Castillo et al., 2016; Kitchlu & Rosner, 2019; Lassen et al., 1995). Hyponatraemia in cancer patients is most often due to either the syndrome of inappropriate antidiuretic hormone secretion, alteration of intravascular volume or medication induced (Kitchlu & Rosner, 2019). The lower sodium concentrations observed in the tumour-bearing dogs with metastasis in this study were mild and not of the same severity reported in people with cancer (Kitchlu & Rosner, 2019). Hyponatraemia associated with tumours in dogs is rarely reported and has been associated with destruction of the adrenal glands by primary or metastatic tumours and subsequent hypoaldosteronism (Beguin et al., 2020; Kook et al., 2010; Merino-Gutierrez et al., 2020). Of the 25 tumour-bearing dogs with metastasis and without intracavitary haemorrhage in this study, only one had metastasis to the adrenal gland, and five dogs had secondary or tertiary tumours of the adrenals. These five dogs had sodium values between 141 and 146 mmol/L (data not shown), and therefore do not explain the lower sodium in dogs with metastasis; thus, the true cause requires further investigation. Sodium was identified through multivariable analysis to be associated with metastasis, with moderate sensitivity and specificity. However, due to overlap

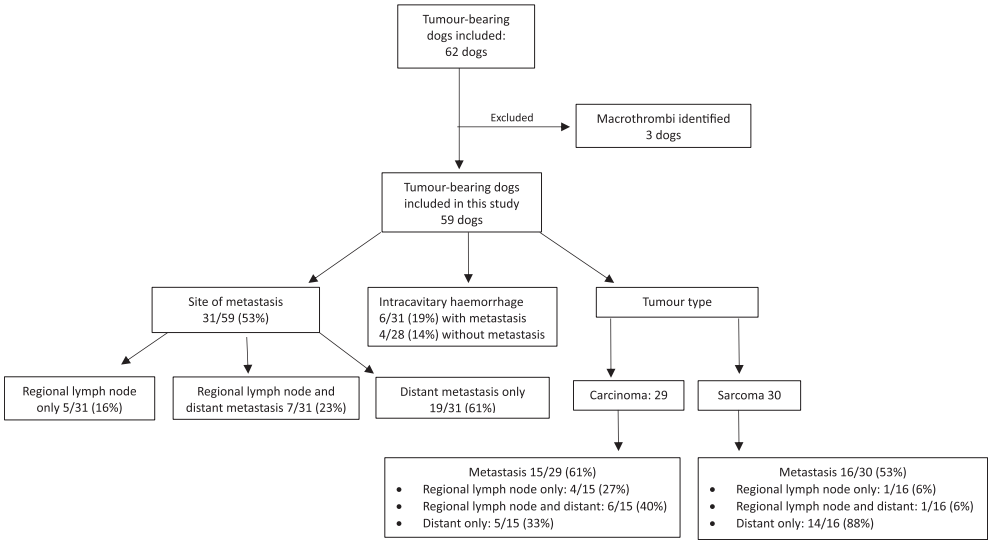

FIG. 1. The tumour-bearing study population's distribution of metastatic status, intracavitary haemorrhage and tumour type.

Table 2. Comparison of haematocrit, platelets, biochemistry and haemostatic variables between tumour-bearing dogs, excluding those with intracavitary haemorrhage, with and without metastasis of the primary tumour

Variable	Tumour-bearing dogs with metastasis Descriptive statistics[†]	Tumour-bearing dogs without metastasis Descriptive statistics[†]	P value*	Institutional reference interval
Haematocrit and platelets				
	n = 25	**n = 24**		
Haematocrit value (L/L)	0.40 (0.30, 0.46)	0.39 (0.33, 0.44)	0.756	0.37 to 0.55
Platelets (×10⁹/L)	382 (302, 586)	404 (256, 590)	0.711	200 to 500
Biochemistry				
Total serum protein (g/L)	62.2 (10.6)	62.6 (6.98)	0.900	56 to 73
Albumin (g/L)	27.7 (5.78)	29.1 (6.03)	0.412	28 to 41
Globulin (g/L)	34.6 (12.7)	33.5 (6.61)	0.712	20 to 41
A:G ratio	0.93 (0.41)	0.92 (0.31)	0.896	0.6 to 1.7
Alanine aminotransferase (U/L)	64.7 (136)	93.6 (193)	0.548	9 to 73
Alkaline phosphatase (U/L)	188 (180)	241 (434)	0.579	20 to 165
Urea (mmol/L)	6.44 (4.29)	5.73 (2.82)	0.496	2.3 to 8.9
Creatinine (μmol/L)	71.3 (36.2)	69.3 (23.9)	0.815	40 to 111
Sodium (mmol/L)[‡]	141 (139, 144)	143 (142, 146)	0.017	142 to 151
Potassium (mmol/L)	4.63 (0.60)	4.55 (0.63)	0.670	3.6 to 5.1
Ionised calcium (mmol/L)[‡]	1.24 (0.06)	1.30 (0.07)	0.002	1.1 to 1.4
C-reactive protein (mg/L)	78.7 (42.8, 160)	58.0 (31.6, 111)	0.638	0 to 15
Serum amyloid A (mg/L)	38.9 (11.4, 122)	22.6 (9.17, 161)	0.623	–
Haemostasis				
	n = 22	**n = 23**		
TEG R time (min)	3.77 (0.61)	3.65 (0.78)	0.554	1.8 to 7.3
TEG K (min)	1.2 (0.8, 1.4)	1.1 (0.9, 1.3)	0.927	1 to 3.6
TEG angle (degrees)	73.7 (70.2, 78.0)	74.5 (69.2, 77.0)	0.982	49.1 to 74.5
TEG MA (mm)	75.0 (68.5, 78.1)	77.0 (66.8, 80.4)	0.474	48.9 to 74.3
TEG G (dyn/cm)	15.0 (10.9, 17.8)	16.7 (10.1, 20.5)	0.467	4.7 to 14.4
TEG lysis 30 (%)[‡]	0 (0, 0.1)	0.5 (0, 1.7)	0.007	0 to 2.4
TEG lysis 60 (%)[‡]	0.45 (0.1, 1.0)	2.5 (1.0, 4.7)	0.004	0 to 8
	n = 23	**n = 24**		
Fibrinogen (g/L)	6.30 (4.51, 8.60)	8.34 (5.01, 9.66)	0.237	1.5 to 4.5
Factor X activity (%)	73.5 (19.8)	84.2 (24.9)	0.110	n/a
Factor VII activity (%)	76.4 (33.3)	88.7 (34.8)	0.224	n/a
D-dimer (ng/mL)	770 (542, 4160)	578 (366, 933)	0.065	0 to 575
Antithrombin activity (%)	81.4 (10.7)	86.6 (13.7)	0.154	100

[†]Presented as the mean (standard deviation) for normally distributed data and the median (interquartile range) for non-normal data
*Based on Student's *t*-tests for normally distributed data and Mann–Whitney *U*-tests for non-normal data
[‡]Significant difference between groups ($P < 0.05$)
A:G albumin to globulin ratio, MA maximum amplitude, n/a not applicable, R reaction, TEG thromboelastogram

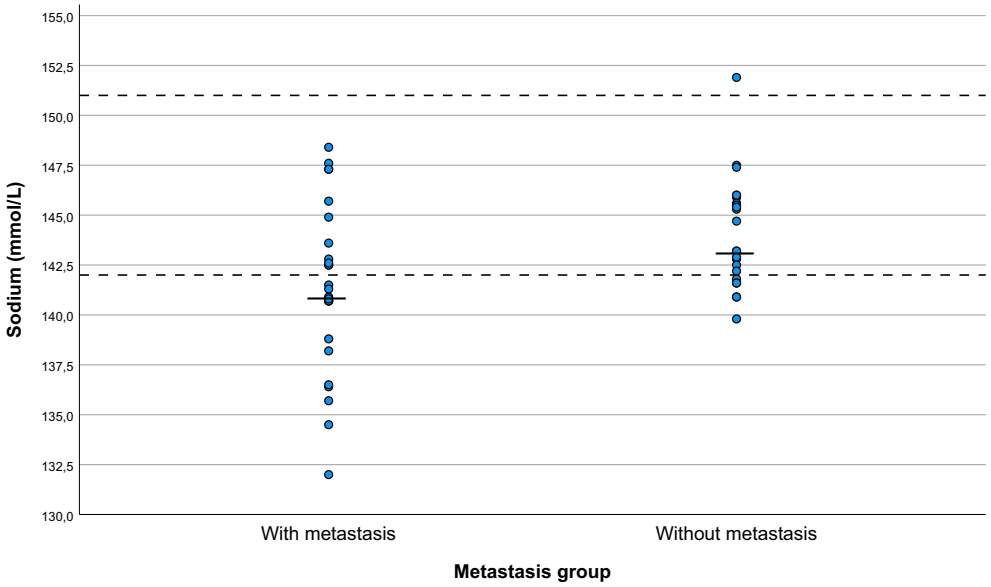

FIG. 2. Scatterplot of sodium in dogs with carcinoma or sarcoma with or without metastasis. Dogs with intracavitary haemorrhage were excluded. Sodium was significantly lower in dogs with metastasis compared to dogs without metastasis (P = 0.017). Medians are represented by a solid line within the scatterplots. Institutional reference intervals are represented by the horizontal dashed lines (142 and 151 mmol/L).

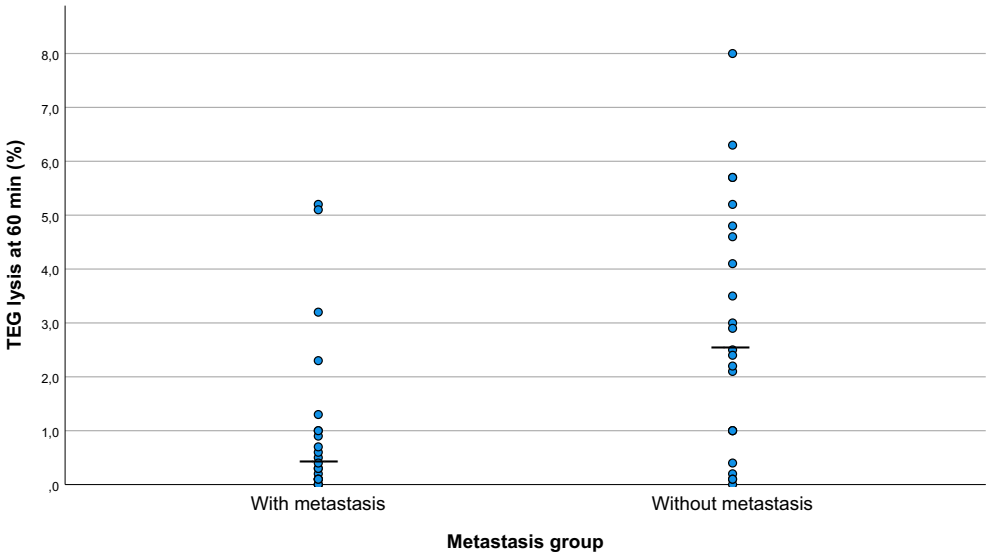

FIG. 3. Scatterplot of thromboelastography-derived lysis at 60 min in dogs with carcinoma or sarcoma with or without metastasis. Dogs with intracavitary haemorrhage were excluded. Lysis at 60 minutes was significantly lower in dogs with metastasis compared to dogs without metastasis (P = 0.017). Medians are represented by a solid line within the scatterplots.

in sodium values in tumour-bearing dogs with and without metastasis, lower sodium (<142.5 mmol/L) should only highlight possible metastasis and the indication for further imaging prior to surgery to determine the presence of metastasis in a dog diagnosed with carcinoma or sarcoma.

The significantly lower iCa in the tumour-bearing dogs with metastasis compared to those without metastasis was an unexpected finding. Mild hypocalcaemia is associated with many cancer types in people, but is not considered a metastatic or prognostic indicator (Ferraz Goncalves et al., 2019). Hypocalcaemia in people is most commonly a complication of bone-modifying agents used as a preventative for bone metastases (White et al., 2020), or in patients after thyroidectomy (Kazaure et al., 2021). In dogs diagnosed with cancer, hypocalcaemia has most commonly been identified post-operatively after removal of parathyroid carcinomas (Erickson et al., 2021), and has not been previously described as an indicator of metastasis. One study reported a significantly lower total calcium in dogs with mammary tumours compared to controls. The suspected underlying cause was calcitonin secretion by some mammary tumours or hypoalbuminaemia affecting total calcium (Duda et al., 2017). Based on our study population, if an iCa of ≤1.24 mmol/L is identified, further diagnostic imaging would be suggested to identify metastasis in dogs with carcinoma or sarcoma.

Table 3. Comparison of haematocrit, platelets, biochemistry and haemostatic variables between tumour-bearing dogs, including those with intracavitary haemorrhage, with and without metastasis of the primary tumour

Variable	Tumour-bearing dogs with metastasis Descriptive statistics†	Tumour-bearing dogs without metastasis Descriptive statistics†	P value*	Institutional reference interval
Haematocrit and platelets				
	n = 31	**n = 28**		
Haematocrit value (%)	0.34 (0.25, 0.45)	0.39 (0.30, 0.44)	0.548	0.37 to 0.55
Platelets ($\times 10^9$/L)	336 (224, 512)	344 (211, 507)	0.970	200 to 500
Biochemistry				
Total serum protein (g/L)	59.40 (11.60)	61.44 (7.35)	0.430	56 to 73
Albumin (g/L)	27.8 (5.66)	29.9 (6.04)	0.170	28 to 41
Globulin (g/L)	31.6 (13.0)	31.6 (7.8)	0.976	20 to 41
A:G ratio	1.04 (0.46)	1.04 (0.42)	0.961	0.6 to 1.7
Alanine aminotransferase (U/L)	130.3 (302.5)	87.9 (179.3)	0.520	9 to 73
Alkaline phosphatase (U/L)	209.9 (190.7)	215.1 (406.2)	0.950	20 to 165
Urea (mmol/L)	7.60 (6.26)	6.11 (2.93)	0.256	2.3 to 8.9
Creatinine (µmol/L)	78.48 (49.06)	76.14 (32.91)	0.832	40 to 111
Sodium (mmol/L)‡	140.9 (138.5, 144.2)	143.2 (141.7, 145.6)	0.028	142 to 151
Potassium (mmol/L)	4.59 (0.56)	4.62 (0.67)	0.863	3.6 to 5.1
Ionised calcium (mmol/L)‡	1.23 (0.07)	1.29 (0.07)	0.001	1.1 to 1.4
C-reactive protein (mg/L)	65.04 (39.16, 140.34)	52.02 (39.19, 95.86)	0.579	0 to 15
Serum amyloid A (mg/L)	41.2 (12.7, 126.1)	22.9 (9.9, 78.9)	0.382	–
Haemostasis				
	n = 28	**n = 27**		
TEG R time (min)	3.81 (1.00)	3.52 (0.83)	0.250	1.8 to 7.3
TEG K (min)	1.2 (0.9, 1.8)	1.2 (1.0, 1.6)	0.932	1 to 3.6
TEG angle (degrees)	72.8 (63.7, 77.7)	72.7 (67.5, 76.1)	0.730	49.1 to 74.5
TEG MA (mm)	74.1 (59.7, 77.3)	74.2 (61.8R, 80.2)	0.341	48.9 to 74.3
TEG G (dyn/cm)	14.3 (7.4, 17.1)	14.4 (8.1, 20.3)	0.333	4.7 to 14.4
TEG lysis 30 (%)‡	0 (0, 0.1)	0.4 (0, 1.7)	0.009	0 to 2.4
TEG lysis 60 (%)‡	0.300 (0.1, 1.00)	2.4 (0.50, 4.70)	0.006	0 to 8
	n = 29	**n = 28**		
Fibrinogen (g/L)	5.20 (3.29, 8.23)	7.40 (4.00, 9.50)	0.148	1.5 to 4.5
Factor X (%)‡	65.8 (26.3)	81.7 (24.7)	0.023	n/a
Factor VII (%)	69.29 (34.05)	87.08 (33.23)	0.051	n/a
D-dimer (ng/mL)‡	2890 (595, 6000)	655 (422, 2433)	0.026	0 to 575
Antithrombin (%)‡	76.94 (15.79)	85.4 (13.55)	0.036	100

†Presented as the mean (standard deviation) for normally distributed data and the median (interquartile range) for non-normal data
*Based on Student's *t*-tests for normally distributed data and Mann–Whitney *U*-tests for non-normal data
‡Significant difference between groups ($P < 0.05$)
A:G albumin to globulin ratio, MA maximum amplitude, n/a not applicable, R reaction, TEG thromboelastogram

Cancer leads to coagulation activation and microthrombosis, which favours tumour progression and survival of metastatic cells (Evans et al., 2017; Kwaan & Lindholm, 2019). Experimental models in mice have demonstrated that absolute depletion of platelets or absent fibrinogen activation reduces the formation of tumour metastases (Camerer et al., 2004; Palumbo et al., 2002). Tumour cells coated in platelets protect tumour cells from host natural killer cells (Medina et al., 2006; Nieswandt et al., 1999; Palumbo et al., 2005; Tesfamariam, 2016), reduce shear stress from blood flow and assist in tumour cell adhesion to the vessel wall and extravasation (Labelle et al., 2011; Schumacher et al., 2014). Additionally, increased tissue factor expression by various tumours has been reported to correlate with a hypercoagulable state and enhanced tumour cell metastasis (Henke et al., 1996; Im et al., 2004; Nierodzik & Karpatkin, 2006; Palumbo et al., 2000, 2005; Remiker & Palumbo, 2018).

In people, a TEG-based hypercoagulable state was associated with regional lymph node metastasis in pancreatic adenocarcinoma (Fleming et al., 2018), a potential predictor of the stage of lung cancer in people (Zhou et al., 2020), and has been linked to cancer progression (Arce et al., 2019). In contrast, rotational thromboelastometry was not able to predict metastasis in gastrointestinal, respiratory and a variety of other tumours in people (Akay et al., 2009). Significant differences have not been previously identified in any TEG variables to differentiate dogs with or without metastasis in various carcinomas (Saavedra et al., 2011), or in dogs with a variety of tumours (Andreasen et al., 2012). One study did report that in dogs with malignant tumours, 50% were hypercoagulable and 17% hypocoagulable, although all hypocoagulable dogs showed signs of bleeding and may have been in overt DIC (Kristensen et al., 2008). In the same cohort of dogs as this study, no significant difference was identified in haemostasis variables between dogs with carcinoma and sarcoma (Pazzi et al., 2023). In the current study, consistent with previous studies in dogs, most TEG variables did not differentiate tumour-bearing dogs with metastasis from those without metastasis, with the exception of significantly decreased Ly30 and Ly60 in dogs with metastasis.

Thromboelastography-based fibrinolysis, represented by Ly30 and Ly60, is reported to be insensitive for the detection of the degree of fibrinolysis in dogs with various sarcomas (Langhorn et al., 2021). Hypofibrinolysis is reported to be partly due to

Table 4. Sensitivity and specificity for specific cut-offs, derived from receiver operator curve analysis for sodium and thromboelastogram-derived lysis at 60 min (TEG Ly60), for the prediction of the presence of metastasis in dogs with carcinoma or sarcoma

Cut-off	Sensitivity % (95% CI)	Specificity % (95% CI)
Sodium (mmol/L)		
<140.5	28 (10 to 46)	96 (88 to 100)
<142.5	64 (45 to 82)	63 (44 to 81)
<144	76 (59 to 93)	46 (26 to 66)
<146	84 (70 to 98)	21 (5 to 37)
TEG Ly60 (%)		
<0.35	46 (25 to 66)	83 (67 to 98)
<1.0	68 (49 to 88)	78 (61 to 95)
<2.5	86 (72 to 100)	52 (32 to 73)
<4.00	91 (79 to 100)	35 (15 to 54)
Sodium < 141.6 mol/L and TEG Ly60 (%) < 2.1		
Series interpretation	50 (30, 70)	96 (80, 100)
Parallel interpretation	91 (73, 98)	57 (36, 75)

decreased tissue plasminogen activator activity and increased plasminogen activator inhibitor-1 activity (PAI-1) (Sawaya et al., 1991; Walsh et al., 2019). Significantly lower PAI-1A activity was previously reported in dogs with carcinoma compared to healthy dogs, although significance was not identified between dogs with and without metastasis (Saavedra et al., 2011).

The combination of a hypercoagulable state with hypofibrinolysis in people with cancer is termed malignancy-associated coagulopathy (Walsh et al., 2019). The previously reported hypercoagulable state of the same cohort of dogs as this study (Pazzi et al., 2023) and reduced TEG-based fibrinolysis in dogs with metastasis is comparable to malignancy-associated coagulopathy in people. Multivariable analysis identified decreased Ly60 to be associated with metastasis in this study, with a moderate sensitivity and specificity. Reduced fibrinolysis would favour microthrombi persistence and the subsequent survival of tumour cells within a fibrin-platelet mesh (Labelle et al., 2011; Medina et al., 2006; Nieswandt et al., 1999; Palumbo et al., 2005; Schumacher et al., 2014; Tesfamariam, 2016). The number of dogs with microthrombi in this study was almost evenly distributed between those with and without metastasis, and the microthrombi previously reported could not be associated with decreased fibrinolysis (Pazzi et al., 2023) or concurrent metastasis in this study. The cause and metastatic effect of lower TEG-based fibrinolysis in the dogs with metastasis in this study is unclear. Greater differences in fibrinolysis in tumour-bearing dogs with and without metastasis are more likely to have been evident if tissue plasminogen-activated TEG or tissue plasminogen activator activity and PAI-1 activity were measured. Although the availability of TEG is limited and overlap between variables existed, the high specificity with serial interpretation and high sensitivity with parallel interpretation of sodium <141.6 mmol/L and Ly60 < 2.1% could be used clinically to pursue the investigation of metastasis prior to surgery.

Interestingly, in tumour-bearing dogs without intracavitary haemorrhage, D-dimer concentrations were not significantly different between dogs with and without metastasis, in spite of the increased TEG-based fibrinolysis in dogs without metastasis. However, the sensitivity and specificity of D-dimer concentration to detect fibrinolysis have not been evaluated (Langhorn et al., 2021), and while the human assay is widely used in veterinary medicine, it has not been fully validated for canines (Goggs et al., 2018; Lynch et al., 2024; Sotos et al., 2023). The D-dimer concentration in dogs with metastasis in this study is in contrast to other studies where increased D-dimer concentration was associated with circulating tumour cells, metastasis and survival in people with specific cancers, as well as metastasis in a variety of cancers in dogs (Andreasen et al., 2012; Chen et al., 2016; Diao et al., 2017; Dirix et al., 2002; Kristensen et al., 2008; Liu et al., 2014; Tas et al., 2012; Tas, Karabulut, et al., 2013; Tas, Kilic, et al., 2013). The equal distribution of dogs with microthrombi in the groups with or without metastasis might explain the comparative lack of raised D-dimer in the non-metastatic group.

Lower factor VII, X and AT activities and higher D-dimer concentrations in dogs with metastasis were only evident when dogs with intracavitary haemorrhage were included in the analysis between tumour-bearing dogs with and without metastasis. The distribution of dogs with haemorrhage was higher in dogs with metastasis (21%) compared to those without metastasis (14%) and a larger number of dogs with haemorrhage would explain the reduced procoagulant and anticoagulant factor activities, and increased D-dimer concentration in dogs with metastasis. Additionally, dogs with metastasis may have been in a more advanced stage of overt DIC.

Due to the risk of malignancy-associated coagulopathy, various anticoagulant therapies have shown promise for improved survival in people with cancer, although it is not always clear if the improved survival is due to the reduced thromboembolic events, reduced metastasis or both. In humans, decreased metastasis or prolonged survival has been shown with therapy with warfarin, long-term aspirin or low molecular weight heparin in different tumour types. (Altinbas et al., 2004; Chiasakul & Zwicker, 2021; Icli et al., 2007; Klerk et al., 2005; Liu et al., 2009; Rothwell et al., 2010; Rothwell et al., 2012; Takiuchi et al., 2018; Zacharski et al., 1984). Given the important association between haemostasis and metastasis, the effects of anticoagulant therapy in metastasis and survival in tumour-bearing dogs are an essential avenue of future research.

Limitations of this study include the relatively low number of dogs in the two tumour-bearing groups. The variables mentioned as markers of metastasis in people are associated with a specific type of cancer, and the grouping of heterogeneous tumour types in this study might have prevented identification of indices of metastasis. Unfortunately, case numbers were too low to allow statistical comparison of tumour sub-types or specific tumour types with and without metastasis separately. Despite thorough post-mortem examination, it is possible that metastasis smaller than 1cm^3 may have been missed as advanced imaging was not performed. Inflammation and corticosteroids have been shown to alter some biochemical parameters and favour a hypercoagulable state (Pazzi et al., 2023; Rose et al., 2011), and non-steroidal inflammatory drugs may affect platelet function (Mullins et al., 2012). Inflammation's role in driving haemostasis changes was previously described

in this cohort of dogs (Pazzi et al., 2023). The prevalence of inflammation was high, with C-reactive protein and serum amyloid A not significantly different between dogs with and without metastasis in this study. Additionally, the number of dogs receiving either medication was low. The effect of inflammation and medication on the dogs cannot be determined as this study was not designed to investigate the effect of these variables in dogs with cancer. Finally, the study design did not allow for identification of causality and whether haemostasis changes encourage metastasis or the inverse. Prospective studies with larger groups are required to validate the findings of this study and evaluate specific carcinomas and sarcomas.

In conclusion, lower sodium and decreased fibrinolysis demonstrated moderate sensitivity and specificity for the identification of metastasis in tumour-bearing dogs without intracavitary haemorrhage. Sodium and fibrinolysis likely have clinical value as screening tests, especially when used in parallel, that raise a clinical suspicion of the possibility of metastasis. The interplay between haemostasis and metastasis is clear from the literature and substantiated in the current study. Whether inhibition of the coagulation cascade would prevent or reduce metastasis requires further evaluation in dogs.

Author contributions

P. Pazzi: Conceptualization (lead); data curation (lead); formal analysis (equal); funding acquisition (supporting); investigation (lead); methodology (lead); project administration (lead); resources (equal); visualization (equal); writing – original draft (lead); writing – review and editing (lead). **G. T. Fosgate:** Data curation (supporting); formal analysis (supporting); methodology (supporting); software (supporting); validation (equal); writing – review and editing (supporting). **A. T. Kristensen:** Conceptualization (supporting); methodology (supporting); supervision (supporting); visualization (equal); writing – review and editing (supporting). **A. Goddard:** Conceptualization (supporting); data curation (supporting); formal analysis (supporting); funding acquisition (lead); investigation (supporting); methodology (supporting); project administration (supporting); resources (supporting); supervision (lead); visualization (supporting); writing – review and editing (supporting).

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Conflict of interest

The consumable items used for the study for the serum amyloid A assays were sponsored by Eiken Chemical Co (Tokyo, Japan).

Disclaimer

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Supporting Information.

Table S1. Signalment of tumour-bearing dogs with carcinoma or sarcoma included in the study.

Table S2. Primary carcinoma or sarcoma and concurrent secondary or tertiary tumours and concurrent inflammation in the dogs included in the study.

Table S3. Variables with P value < 0.2 after univariate analysis for the prediction of the presence of metastasis in dogs with carcinoma or sarcoma entered into multivariable analysis.