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A prospective evaluation of the prevalence of thromboemboli and associated hemostatic dysfunction in dogs with carcinoma or sarcoma

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Abstract

Background: Knowledge of the prevalence of thromboemboli and the associated hemostatic status in dogs with carcinoma or sarcoma is unknown and might allow earlier intervention.

Objectives: Estimate prevalence of thromboemboli and their association with hemostatic changes in dogs with carcinomas or sarcomas; estimate predictive values of hemostatic variables for thromboembolic disease in tumor-bearing dogs.

Animals: Thirty-two dogs with sarcoma, 30 with carcinoma, 20 healthy age-controlled dogs.

Methods: Prospective cross-sectional study. A hemostasis panel (platelet concentration, thromboelastography, fibrinogen and D-dimer concentration, factor X, VII and antithrombin activity) was performed in all dogs. Tumor-bearing dogs underwent complete post mortem and histopathological evaluation. Comparisons between healthy dogs and tumor-bearing dogs with and without intracavitary hemorrhage; and tumor-bearing dogs with and without microthrombi were analyzed.

Results: Thromboembolic disease was identified in 32/62 (52%, 95% CI: 39%-65%) tumor-bearing dogs. Microthrombi were identified in 31/62 (50%, 95% CI: 37%-63%) dogs, 21/31 (68%, 95% CI: 49%-83%) had exclusively intra-tumoral microthrombi, 10/31 (32%, 95% CI: 17%-51%) had distant microthrombi. Macrothrombi were identified in 3 tumor-bearing dogs. Hemostatic changes potentially consistent with overt and non-overt disseminated intravascular coagulation were identified in some tumor-bearing dogs. D-dimer concentrations were significantly higher ($P = .02$) and platelet concentration significantly lower ($P = .03$) in tumor-bearing dogs with microthrombi compared to tumor-bearing dogs without microthrombi. D-dimer concentration above 500 ng/mL was 80% sensitive and 41% specific for the prediction of microthrombi presence.

Abbreviations: AT, antithrombin; 95% CI, 95% confidence interval; CRP, C-reactive protein; G, global clot strength; K, time for the tracing to achieve a predetermined clot strength; MA, maximum amplitude; OR, odds ratio; R, reaction time; RDW, red cell distribution width; SAA, serum amyloid A; TEG, thromboelastography; VTE, venous thromboemboli.

Conclusion: The high microthrombi prevalence and concomitant hemostatic dysfunction in dogs with carcinomas or sarcomas has not previously been reported, though the clinical importance is unknown. Increased D-dimer concentration might increase suspicion of microthrombi.

KEYWORDS

cancer, hemangiosarcoma, hemostasis, macrothrombi, thromboelastography

1 | INTRODUCTION

Clinical evidence of thromboembolism is seen in 10% to 20% of people with cancer,[1-5] and is the second most common cause of death in human cancer patients.[6] Cancer has also been recognized as one of the most common causes of thrombosis in dogs.[7-9] The prevalence of thromboembolic disease was reported as low as 2.6% in a retrospective study of dogs with solid tumors.[10]

Among the hemostatic abnormalities described in dogs with cancer,[11-13] hypercoagulability, based on thromboelastography (TEG) evaluation, is one of the most common, but hypocoagulability and disseminated intravascular coagulation (DIC) are also reported.[11,14,15] A combination of procoagulant activation, inhibitor consumption, and increased fibrinolytic activity characterizes DIC.[16-18] The continuum of DIC in cancer involves systemic activation of coagulation with subsequent generation of intravascular fibrin leading to micro-thrombosis of small blood vessels. Non-overt DIC is described as mild to moderate hemostatic changes without hemorrhage or clinical evidence of organ dysfunction.[16,19,20] Overt DIC might develop with continued excessive activation of coagulation and consumption of inhibitors, resulting in severe hemostatic changes and eventually organ dysfunction and hemorrhage.[16,19,20]

Microthrombi are aggregates of fibrin, red blood cells, and platelets within blood vessels that remain after tissue processing,[10] are thought to be integral to the development of macrothrombi, cause substantial vascular injury,[21-23] and play a role in the seeding and survival of metastatic tumor cells.[24,25] Intra-tumoral microthrombi have previously been described in dogs with various solid tumors.[10,26-28]

The most clinically available and widely investigated thromboembolic disease diagnostic laboratory test is D-dimer concentration.[29-31] D-dimer concentration has been reported to have a high sensitivity but low specificity for the diagnosis of venous thromboemboli (VTE) in people,[29-32] and macro-thromboembolic disease in dogs.[33-35] Clinical confirmation of thromboembolic disease relies on diagnostic imaging that can lack sensitivity.[36] The gold standard for the detection of thromboemboli remains macroscopic and microscopic post mortem examination.[37] Despite carcinomas and sarcomas being commonly diagnosed in dogs,[10,38,39] the prevalence of thromboemboli, and diagnostic use of associated hematological, inflammatory (represented by acute phase proteins [APP]) and hemostatic changes have not been investigated in dogs with these cancers.

The aims of this prospective study were to (a) estimate the prevalence of thromboembolic disease, specifically microthrombi, in dogs with carcinoma or sarcoma; (b) identify differences in the hematological, inflammatory and hemostatic variables between healthy age-controlled dogs and tumor-bearing dogs with and without intracavitary hemorrhage; as well as between tumor-bearing dogs (without intracavitary hemorrhage) with and without microthrombi; and (c) identify variables predictive of the presence of microthrombi in tumor-bearing dogs. We hypothesized the prevalence of microthrombi would be higher than previously reported; measured variables would indicate that a coagulopathy, specifically DIC, is present and likely contributes to microthrombi formation; and D-dimer concentration would be increased in dogs with microthrombi.

2 | MATERIALS AND METHODS

2.1 | Study design

This prospective, cross-sectional study included client-owned dogs presented to the Onderstepoort Veterinary Academic Hospital, University of Pretoria between December 2018 and September 2020. Research ethics approval was granted by the Research Ethics Committee of the Faculty of Veterinary Science, University of Pretoria (REC 105-18) and Animal Ethics Committee of the University of Pretoria (V100-18). Informed owner consent was obtained for all dogs included in this study.

Dogs were eligible for inclusion if they were diagnosed with either a carcinoma or sarcoma, based on clinical findings and cytological evaluation, were older than 1 year of age, weighed greater than 5 kg and had no clinical evidence of concurrent disease. Dogs were included if owners decided to euthanize based on a poor prognosis as a result of the primary tumor at the time of presentation, or due to financial constraints preventing curative surgery or treatment. The primary tumor of tumor-bearing dogs was defined as the tumor for which the dog was presented and the reason for subsequent euthanasia. Dogs were excluded if at the time of blood collection there was evidence of (a) bite wounds, trauma or inflammatory conditions identified on clinical examination; (b) treatment with any medication known to alter hemostasis in the 2 weeks before sample collection with 2 exceptions—dogs that had received non-steroidal anti-inflammatory drugs or prednisolone were included; or (c) another tumor type identified during clinical examination.

In addition to tumor-bearing dogs, client or staff owned dogs were included as healthy, age-controlled unaffected dogs. The healthy unaffected dogs had to be older than 9 years of age, weigh more than 5 kg, have no evidence of concurrent disease, trauma or tumors, and

received no treatment with any medication known to alter hemostasis for at least 4 weeks before enrolment. They were considered healthy based on lack of history of illness for 4 weeks before enrolment, unremarkable physical examination, peripheral blood smear, fecal flotation, abdominal ultrasound, and 3-view thoracic radiographs.

2.2 | Sample collection and diagnostic testing

Before euthanasia of the tumor-bearing dogs, blood samples were collected in order—serum, 3.2% sodium citrate, and EDTA tubes (Beckton Dickinson, UK) via jugular venipuncture with a 21-gauge needle, using vacuum assistance as previously described.[40] Time between sample collection and euthanasia, and time between euthanasia and post mortem examination was recorded. The same sampling technique was used for blood collected from the unaffected dogs. No healthy unaffected dogs were euthanized for this study.

A CBC was performed within 2 hours of collection, using the EDTA sample, on an Advia 2120i (Siemens, Berlin Germany). Manual differential leukocyte counts were also performed. Serum was separated by centrifugation and stored at −80°C for batch analysis of APPs, specifically C-reactive protein (CRP; Gentian, Norway) and serum amyloid A (SAA; Eiken, Japan) on the Cobas Integra 400 plus analyzer (Roche, Basel, Switzerland).

The sodium citrate sample was used to evaluate hemostatic variables. Thromboelastography was performed 30 minutes after collection using kaolin as the activator, on the TEG 5000 Thrombelastograph (Haemonetics Corporation, Massachusetts, USA), according to the manufacturer's instructions. Variables derived from the thromboelastogram included reaction time from 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). The individual dog thromboelastogram-derived variables were evaluated and statistically compared as groups. A group with a hypercoagulable thromboelastogram was defined as 2 or more of a combination of significantly higher α-angle, MA, G or lower R or K compared to the group of healthy unaffected dogs. A group with a hypocoagulable thromboelastogram was defined as 2 or more of a combination of significantly lower α-angle, MA, G or higher R or K compared to the healthy unaffected dogs.[41] The citrated plasma was removed after centrifugation and stored at −80°C for batch analysis of antithrombin (AT) activity (Precimat Chromogen, Roche, Switzerland) on a Cobas Integra 400 plus analyzer; D-dimer and fibrinogen concentrations and factors VII (FVII) and X activity (HemosIL, Ilex, South Africa), using the ACL Elite Analyzer (Instrumentation laboratories, Massachusetts, USA). For AT activity, a pooled control sample, consisting of 10 healthy dogs, was run with each batch of tests and all dog samples were normalized against the control. For FVII and FX activity, the coagulation factor activity of the tumor-bearing dogs was normalized against the means of the healthy unaffected dogs' FVII and FX activity, which was presumed to have 100% activity.

For this study, the diagnosis of DIC in tumor-bearing dogs was based on the International Society of Thrombosis and Hemostasis criteria,[16] previously validated in dogs.[18] Criteria for non-overt DIC included significant changes in procoagulant activation (lower coagulation factor activity, hypercoagulable thromboelastogram[42]), inhibitor consumption (lower AT activity) and increased fibrinolytic activity (higher D-dimer concentration) when compared to healthy unaffected dogs, and without concurrent evidence of intracavitary hemorrhage. For the diagnosis of overt DIC in tumor-bearing dogs the criteria were the same, except for a hypocoagulable thromboelastogram, or a significantly lower platelet concentration compared to the healthy unaffected dogs, and evidence of concurrent clinical bleeding, that is, intracavitary hemorrhage.

2.3 | Post mortem examination

All tumor-bearing dogs enrolled in this study underwent a full post mortem examination within 2 hours of euthanasia. Samples of the tumor, draining lymph node (when identified), 1 cm^3 sections of all lobes of parenchymatous organs and any macroscopically abnormal tissue were placed in formalin (10%) for routine hematoxylin and eosin staining and light microscopic examination, with specific attention to the identification of microthrombi, by a board-certified pathologist. For details of post mortem examination and organ sampling refer to File S1. Macrothrombi were defined as visible and palpable thrombi within macroscopic blood vessels identified during post mortem examination and confirmed on histopathology to be a mesh of cross-linked fibrin protein with varying amounts of aggregated platelets, white blood cells, and red blood cells.[10] Microthrombi were defined as a mesh of cross-linked fibrin protein forming a thrombus-like structure within microscopic blood vessels, adherent to the vessel wall after tissue processing, with varying amounts of aggregated platelets, white blood cells, and red blood cells.[10] Intra-tumoral thrombi were classified as the thrombi identified within the vasculature of the primary tumor. Distant thrombi were classified as distant if they were identified in vasculature in an organ apart from or distant to the primary tumor. If a second or third additional tumor was identified during post mortem examination and was histologically different from the primary tumor, it was defined as a secondary primary tumor or tertiary tumor, respectively.

Additional observations included: location of tumors; presence of macroscopic ulceration or histological evidence of inflammation or necrosis of the tumor; and presence, severity and staging (eg, mitral valve disease[43]) of comorbidities. A comorbidity was defined as a concurrent condition identified during post mortem examination or on histopathology that was unrelated to the primary tumor.

2.4 | Statistical analysis

Data were analyzed using SPSS 27 software (IBM SPSS Inc., Armonk, New York, USA). 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 before statistical analysis. If transformation did not improve the distributional form then nonparametric statistical methods were employed.

The tumor-bearing dogs were divided into carcinoma or sarcoma groups based on the histopathological diagnosis of the primary tumor. Prevalence of thromboembolic disease was calculated as the number of tumor-bearing dogs with thrombi (macrothrombi and microthrombi) divided by the total number of tumor-bearing dogs. Prevalence of microthrombi was determined as the number of tumor-bearing dogs with microthrombi divided by the total number of tumor-bearing dogs. Ninety-five percent confidence interval (95% CI) were calculated for prevalences. Microthrombi were further divided into intratumoral and distant microthrombi for descriptive purposes.

Categorical data were compared between study groups using chi-square tests. Quantitative data normally distributed were compared using Student *t* tests. Quantitative data not normally distributed were compared using Mann-Whitney *U*. Dogs were classified into breed types (eg, working dogs, toy breeds, etc.) for the tumor-bearing dogs and healthy unaffected dogs. Differences in breed, age, and sex distribution between groups were evaluated. Dogs with macrothrombi were excluded from further statistical analysis due to the hemostatic changes associated with development of macrothrombi influencing the interpretation of subsequent comparisons between groups (data reported separately).[44-46] Data were compared between the healthy unaffected dogs and tumor-bearing dogs without intracavitary hemorrhage, and then between healthy unaffected dogs and tumor-bearing dogs with intracavitary hemorrhage. Normal reference ranges have not been established for hematological, APPs or hemostatic variables for older dogs (>9 years of age) and all variables were compared as a group to age-controlled healthy unaffected dogs. Further analysis from this point was performed after dogs with intracavitary hemorrhage were excluded, because of the effect of hemorrhage on hemostatic and other variables.[11,14,33,47,48] Data were compared between carcinomas and sarcomas and between tumor-bearing dogs with and without microthrombi, regardless of the type of primary tumor. The sensitivity and specificity of D-dimer assays for the prediction of thromboembolic disease and DIC at a cut-off value of >500 ng/mL in canine studies have ranged between 87% and 100% and 26% and 70%, respectively.[33,48] Receiver operator characteristic curve analysis was performed to determine the sensitivity and specificity of D-dimer to predict the presence of microthrombi in tumor-bearing dogs at a cut-off value of >500 ng/mL.

The association between laboratory variables and other potential risk factors in tumor-bearing dogs with microthrombi was further evaluated using binary logistic regression. Univariate screening models were fit for each continuous variable and variables with *P* values <.2 were selected for multivariable modeling. The medians of the variables of the tumor-bearing dogs were used to determine their predictive association with microthrombi in univariate analysis. Multivariable models were fit in a backwards stepwise process.

Post hoc analysis was performed using receiver operator characteristic curves to estimate the sensitivity and specificity of significant predictors for the detection of microthrombi. Unless stated otherwise, significance was set as $P < .05$.

3 | RESULTS

3.1 | Study group characteristics

Sixty-two tumor-bearing dogs and 20 healthy unaffected age-controlled dogs were included. Twenty-three breeds were in the tumor-bearing group and 9 in the healthy unaffected group (Table 1), with no significant difference between groups for breed categories, age, or sex. All blood samples were collected <40 minutes before euthanasia, and all post mortem examinations were started within 45 minutes of euthanasia except for 1 (75 minutes).

Thirty primary carcinomas were identified, with half originating in the mammary glands (15/30, 50%). Thirty-two primary sarcomas were identified with visceral hemangiosarcoma (11/32, 34%), the most frequently diagnosed sarcoma (Table 2; Table S1). Intracavitary hemorrhage was identified in 10/62 (16%) dogs. Eight of the 10 dogs (80%) had visceral hemangiosarcomas, 1 hepatic spindle cell sarcoma and 1 hepatocellular carcinoma. A secondary primary tumor was incidentally identified in 25 (40%) dogs, with 10 (40%) secondary primary tumors identified in dogs with primary carcinoma and 15 (60%) secondary primary tumors identified in dogs with primary sarcoma. Tertiary tumors, were identified in 8/62 (13%) dogs (Table S2). Of the 25 dogs with more than 1 primary tumor, 20 (80%) had a secondary or tertiary primary tumor unrelated to the primary tumor tissue lineage (epithelial or mesenchymal).

3.2 | Prevalence of thromboembolic disease in dogs with carcinoma or sarcoma

Thromboembolic disease was identified in 32/62 (52%, 95% CI: 39%-65%) of the tumor-bearing dogs. Three dogs were identified with macrothrombi (Table S1). Two of the dogs with macrothrombi also had distant microthrombi (Table S3). Microthrombi were identified in 31/62 (50%, 95% CI: 37%-63%) of all tumor-bearing dogs. Figure 1 depicts prevalence of thromboembolic disease, the location of microthrombi and the tumor type associated with microthrombi.

For the 30 dogs diagnosed with carcinoma, the majority of microthrombi were of mammary tumor origin 7/13 (54%, 95% CI: 25%-81%), of which 4 (57%, 95% CI: 18%-90%) were intra-tumoral only, 2 (29%, 95% CI: 4%-71%) were intra-tumoral and distant, and 1 (14%, 95% CI: 0%-58%) was distant only. For the 32 dogs diagnosed with sarcoma, the majority of microthrombi were associated with visceral hemangiosarcomas 10/18 (56%, 95% CI: 31%-79%), of which 8 (80%, 95% CI: 44%-98%) were exclusively intra-tumoral and 2 (20%, 95% CI: 3%-56%) were both intra-tumoral and distant.

Of all the tumor-bearing dogs with microthrombi, 8/31 (26%, 95% CI: 12%-45%) had intracavitary hemorrhage. Eight of the 10 (80%, 95% CI: 44%-98%) dogs with intracavitary hemorrhage had microthrombi. Inflammation, ulceration or necrosis of the tumors were identified clinically or on histopathology in 50/62 (81%, 95% CI: 69%-

TABLE 1 Signalment of tumor-bearing dogs with carcinoma or sarcoma and healthy unaffected dogs included in the study.

	Breed (number of dogs per breed)	Median age in years (interquartile range)	Sex
Tumor-bearing dogs	Mixed breed (8) *Working breeds* German shepherd (7), Jack Russel terrier (7), Labrador retriever (7), Boerboel (6), Dachshund (5), Cocker spaniel (2), Fox terrier (2), and 1 each of Beagle, Border collie, Boxer, Bullmastiff, Rottweiler, Scottish terrier *Fighting breeds* American Pitbull terrier (2), Staffordshire bull terrier (2), and 1 Bull terrier *Toy breeds* Pug (2) and one each of Pekingese, Pomeranian, and Standard poodle *Show or companion dogs* One Great Dane and Irish terrier	10.5 years (8.9-13.6)	FI: 21, FS: 17, MN: 15, MI: 9
Healthy unaffected dogs	Mixed breed (8) *Working breeds* Jack Russel terrier (4), Dachshund (2) and one Basset hound and German shepherd *Fighting breeds* One Bull terrier and Staffordshire bull terrier. *Toy breeds* One miniature French poodle and Pekingese	10.25 years (9.25-11.93)	FS: 9, MN: 9, MI: 2

Abbreviations: FI, female intact; FS, female sterilized; MI, male intact; MN, male neutered.

90%) of the tumor-bearing dogs; 24/30 (80%, 95% CI: 61%-92%) of carcinoma dogs and 26/32 (81%, 95% CI: 63%-93%) of sarcoma dogs. Inflammation, ulceration, or necrosis were present in 26/31 (84%, 95% CI: 66%-95%) of dogs with microthrombi and 24/31 (77%, 95% CI: 59%-90%) of dogs without microthrombi. Comorbidities were present in 26/62 (42%, 95% CI: 30%-55%) of tumor-bearing dogs (Tables S1 and S2), of which 9/26 (35%, 95% CI: 17%-56%) dogs had concurrent microthrombi. Concurrent medication of any type was identified in 21/62 dogs (34%, 95% CI: 22%-47%; Tables S1 and S4).

3.3 | Hematological, APP, and hemostatic variables compared between healthy unaffected dogs (n = 20) and tumor-bearing dogs with (n = 10) and without intracavitary hemorrhage (n = 49)

Hemoglobin, red blood cell count, and hematocrit were significantly lower, and neutrophil, monocyte, CRP and SAA concentrations significantly higher in tumor-bearing dogs compared to healthy unaffected dogs (Tables 3 and 4). Compared to the healthy control dogs, the tumor-bearing dogs with and without hemorrhage, had lower FVII, FX, and AT activities; and higher D-dimer concentrations. In addition, tumor-bearing dogs without intracavitary hemorrhage compared to heathy dogs, had significantly higher TEG angle, MA, G, and lower K variables (Tables 3 and 4).

Dogs with intracavitary hemorrhage (n = 10) were excluded and variables were compared in 49 tumor-bearing dogs.

3.4 | Hematological, APP, and hemostatic variables compared between tumor-bearing dogs with carcinomas (n = 28) and sarcomas (n = 21)

Only eosinophil concentration was significantly higher in dogs with sarcomas (Table 5).

3.5 | Hematological, APP, and hemostatic variables compared between tumor-bearing dogs with (n = 21) and without (n = 28) microthrombi

Red cell distribution width, band neutrophil, CRP, and SAA concentrations were significant higher and platelet concentration significantly lower in tumor-bearing dogs with microthrombi (Table 6). The TEG R value and D-dimer concentration (Figure 2) were significantly higher in tumor-bearing dogs with microthrombi. Only 4/21 (19%) dogs with microthrombi had a D-dimer concentration <500 ng/mL. The sensitivity and specificity of D-dimer concentrations of >500 ng/mL for the prediction of the presence of microthrombi was 80% and 41%, respectively (Table 7).

3.6 | Predictors of the presence of microthrombi in tumor-bearing dogs

After univariate analysis (Table S5), multivariable analysis identified lower platelet concentration (odds ratio [OR]: 0.199, 95% CI: 0.04-

TABLE 2 Primary tumors of dogs included in the study, tumor-associated microthrombi location (exclusively intra-tumoral, intra-tumoral, and distant or exclusively distant) and site of distant microthrombi (excludes dogs with concurrent macrothrombi).

Category of tumor	Primary tumor	Exclusively IT thrombi	Both IT & distant thrombi	Exclusively distant thrombi	Site of distant microthrombi
Sarcoma (n = 30)	Subcutaneous soft tissue sarcoma (n = 3)				
	Splenic hemangiosarcoma (n = 3)[a]	2			
	Right atrial hemangiosarcoma (n = 2)[a]	1	1		Lung
	Splenic & right atrial hemangiosarcoma (n = 2)[a]	2			
	Hepatic hemangiosarcoma (n = 2)[a]	1	1		Kidney
	Cutaneous hemangiosarcoma (n = 2)	1[b]			
	Mammary osteosarcoma (n = 2)	1		1	Kidney
	Esophageal osteosarcoma (n = 2)	1			
	Oral osteosarcoma (n = 2)	1			
	Muscle hemangiosarcoma	1			
	Subcutaneous hemangiosarcoma	1[c]			
	Hepatic spindle cell sarcoma[d]	1			
	Cutaneous soft tissue sarcoma	1			
	Muscle soft tissue sarcoma				
	Rib chondroblastic osteosarcoma				
	Mixed mammary sarcoma				
	Splenic stromal sarcoma				
	Mandibular soft tissue sarcoma				
	Mixed peri-orbital anaplastic sarcoma				
Carcinoma (n = 29)	Complex mammary carcinomas (n = 4)	2			
	Simple tubular mammary carcinoma (n = 3)	1[e]	1		Lung
	Mixed mammary carcinoma (n = 2)		1[f]	1	Lung (1), Liver (1)
	Squamous cell carcinoma (n = 2)	1			
	Hepatocellular carcinoma (n = 2)[d]			1	Spleen
	Spindle cell mammary carcinoma	1			
	Pulmonary carcinomatosis	1			
	Carcinoma (papillary & solid)—source unknown	1			
	Tubular mesothelioma			1	Lung
	Simple solid mammary carcinoma				
	Simple tubulopapillary mammary carcinoma				
	Anaplastic mammary carcinoma				
	Mammary ductal carcinoma				
	Mixed type carcinoma & simple tubulopapillary mammary carcinoma				
	Anaplastic cholangiocellular carcinoma				
	Sinonasal transitional carcinoma				
	Pulmonary carcinoma (tubulopapillary type)				
	Solid scirrhous prostatic carcinoma				
	Thyroid microfollicular carcinoma				
	Urothelial carcinoma				
	Apocrine gland adenocarcinoma (anal sac)				

Note: Numbers in each column indicate the number of tumor-bearing dogs identified with microthrombi per category.
Abbreviations: IT, intra-tumoral; n, number of dogs.
[a]Indicates 2 dogs with intracavitary hemorrhage identified at post mortem.
[b]Intra-tumoral microthrombi identified in the primary and secondary primary tumor (indolent lymphoma).
[c]Intra-tumoral microthrombi identified in the primary and secondary primary tumor (thyroid carcinoma).
[d]Indicates 1 dog with intracavitary hemorrhage identified at post mortem.
[e]Intra-tumoral microthrombi identified in the secondary primary tumor (hemangioma).
[f]Intra-tumoral microthrombi identified in the secondary primary and tertiary tumor (compact thyroid carcinoma, adrenal cortical carcinoma).

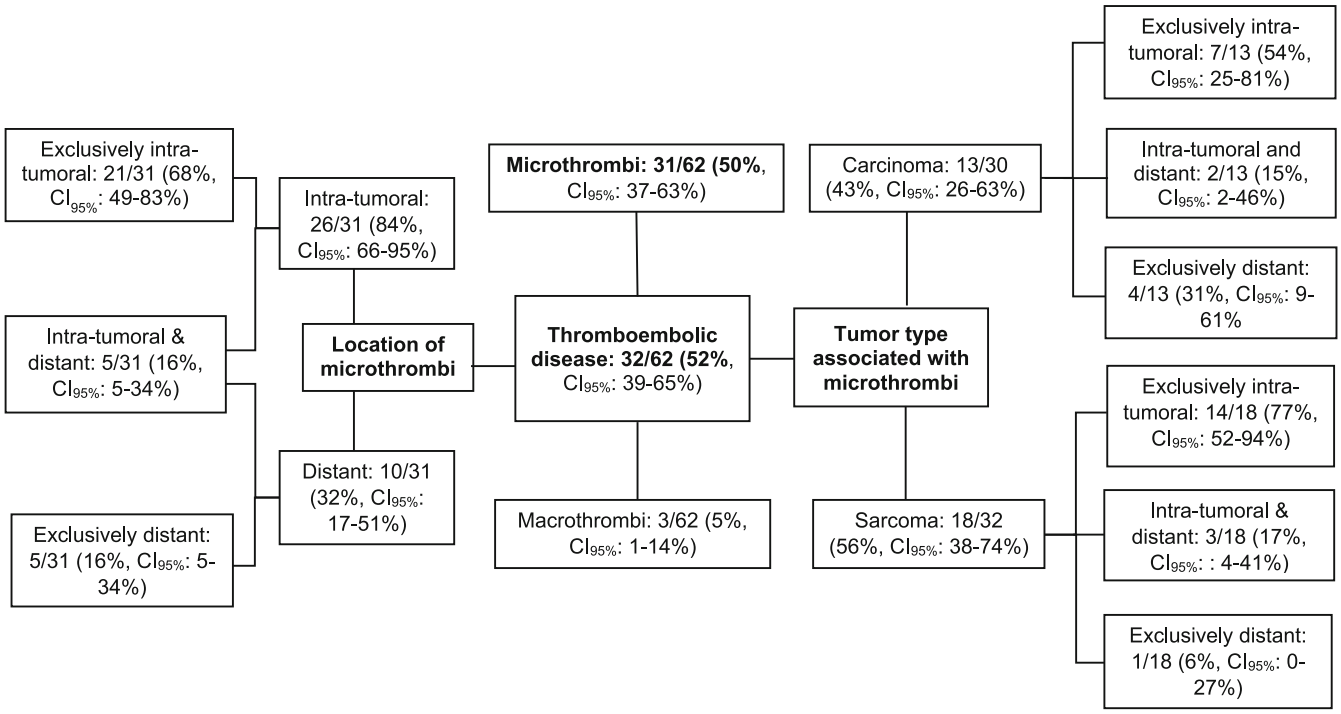

FIGURE 1 The prevalence of thromboembolic disease, the location of microthrombi, and the tumor type associated with microthrombi in tumor-bearing dogs with carcinoma and sarcoma. 95% CI, 95% confidence interval.

0.984; $P = .05$), higher band neutrophil concentration (OR: 15.43, 95% CI: 2.23-106; $P = .006$) and higher R value (OR: 6.21, 95% CI: 1.22-31.7; $P = .03$) as significant predictors for the detection of microthrombi. The median cut-off values and respective sensitivity and specificity for each variable for prediction of the detection of microthrombi were: $<400 \times 10^3/mm^3$, 67% and 61% for platelets ($P = .03$; Table 7); $>0.00 \times 10^3/mm^3$, 52% and 89% for band neutrophils ($P = .01$); and >3.8 minute, 74% and 58% for R ($P = .02$).

4 | DISCUSSION

In this prospective post mortem study, a high prevalence of microthrombi (50%) was identified in dogs diagnosed with carcinoma or sarcoma. The majority of microthrombi were intra-tumoral, but 32% were distant to the primary tumor. The findings of this study identified marked systemic inflammation in tumor-bearing dogs, as well as increased procoagulant activation, inhibitor consumption and fibrinolytic activity potentially indicative of non-overt DIC, and in tumor-bearing dogs with hemorrhage, overt DIC.[16,18] D-dimer concentration above 500 ng/mL was identified as a sensitive indicator for the presence of microthrombi in this cohort of dogs, but lacked specificity.

The overall prevalence for microthrombi in tumor-bearing dogs in this study was 50%, of which 68% were exclusively intra-tumoral, 16% were intra-tumoral and distant, while 16% were exclusively distant. In contrast, a 2.6% prevalence for microthrombi in biopsies or post mortems of 2274 solid tumors was reported in a recent retrospective study.[10] Of the microthrombi reported, 91% were exclusively intra-tumoral and 9% distant. Additionally, the retrospective study reported a prevalence of microthrombi of 70% in sarcoma and 15% in carcinoma and in the current study the prevalence of microthrombi was similar in dogs with sarcoma (56%) and carcinoma (43%).[10] The reason for the large discrepancy in overall prevalence and location of microthrombi between studies is most likely due to the prospective nature of the current study, rapid and systematic post mortem evaluation and sampling, including histological evaluation of each individual organ and consistent reporting of microthrombi that is not feasible retrospectively. The discrepancy in results could possibly also be the result of dogs with more advanced disease being included, thereby increasing the tumor burden, inflammation or necrosis and likelihood of thrombi formation, as in the current study dogs were euthanized due to a poor prognosis or financial constraints that prevented intervention. Additionally, the prevalence of intra-tumoral microthrombi in visceral hemangiosarcomas (32%) and hemangiosarcoma from all locations (36%) in the current study was markedly less than previously reported in hemangiosarcomas from all locations (65%).[10] This is likely, in part, due to the high number of cutaneous hemangiosarcomas with intra-tumoral microthrombi in the retrospective study (28%) compared to the current study (3%).

Local intra-tumoral disturbances in hemostasis, which might result in intra-tumoral microthrombi formation, occur due to disorganized vasculature with altered blood flow and blood pooling, higher tumor tissue factor expression, a pro-inflammatory state of the affected endothelium within the tumor, and upregulation of hemostatic pathways.[7,27,49-52] Microthrombi are considered integral to the formation of macrothrombi.[53] Surprisingly, 1 dog diagnosed with both a portal vein and

TABLE 3 Comparison of hematologic, acute phase proteins, and hemostatic variables between healthy unaffected dogs and tumor-bearing dogs without intracavitary hemorrhage diagnosed with carcinoma or sarcoma (excluding dogs with macrothrombi).

Variable	Healthy unaffected dogs[a]	Tumor-bearing dogs without intracavitary hemorrhage[a]	*P* value[b]
Hematology	n = 20	n = 49	
Hemoglobin (g/dL)[c]	17.7 (17.0, 18.5)	13.1 (9.90, 14.8)	<.001
Red blood cell count ($\times 10^6$/mm^3)[c]	7.82 (7.16, 8.49)	5.67 (4.64, 6.70)	<.001
Hematocrit (%)[c]	0.53 (0.51, 0.56)	0.39 (0.31, 0.44)	<.001
Mean corpuscular volume (fL)	68.6 (66.2, 72.1)	67.9 (66.0, 70.2)	.40
Mean cell hemoglobin concentration (g/dL)	33.4 (32.8, 33.5)	33.0 (32.5, 33.6)	.32
Red cell distribution width (%)	13.6 (13.1, 13.9)	13.9 (13.1, 15.4)	.15
White cell count ($\times 10^3$/mm^3)[c]	8.27 (7.24, 9.80)	16.2 (11.6, 21.6)	<.001
Neutrophils ($\times 10^3$/mm^3)[c]	6.45 (1.84)	15.3 (9.17)	<.001
Band neutrophils ($\times 10^3$/mm^3)	0 (0, 0)	0 (0, 0.26)	.13
Lymphocytes ($\times 10^3$/mm^3)	1.34 (0.53)	1.55 (0.85)	.30
Monocytes ($\times 10^3$/mm^3)[c]	0.41 (0.30, 0.49)	0.97 (0.61, 1.40)	<.001
Eosinophil ($\times 10^3$/mm^3)	0.38 (0.30, 0.55)	0.30 (0.16, 0.58)	.36
Basophil ($\times 10^3$/mm^3)	0 (0, 0)	0 (0, 0.02)	.34
Platelets ($\times 10^3$/mm^3)[c]	261 (222, 300)	400 (288, 586)	<.001
Acute phase proteins	n = 20	n = 49	
C-reactive protein (μg/mL)[c]	<10 (<10, <10)	65.0 (42.8, 142)	<.001
Serum amyloid A (μg/mL)[c]	<2 (<2, <2)	25.9 (9.67, 122)	<.001
Hemostasis	n = 20	n = 45	
TEG R time (min)	3.53 (1.15)	3.71 (0.70)	.44
TEG K (min)[c]	1.8 (1.5, 2.0)	1.1 (0.9, 1.3)	<.001
TEG angle (°)[c]	65.2 (62.1, 69.2)	73.9 (69.6, 77.7)	<.001
TEG MA (mm)[c]	59.7 (58.0, 62.5)	75.3 (67.9, 80.1)	<.001
TEG G (dyn/cm)[c]	7.4 (6.9, 8.4)	15.2 (10.6, 20.1)	<.001
TEG lysis 30 (%)[c]	0 (0, 0)	0.1 (0, 0.6)	.02
TEG lysis 60 (%)	1.10 (0.30, 1.95)	1.0 (0.2, 3.2)	.66
	n = 20	n = 47	
Fibrinogen (mg/dL)[c]	324 (261, 368)	776 (488, 925)	<.001
Factor X activity (%)[c]	100 (16.7)	79.0 (22.9)	<.001
Factor VII activity (%)	100 (32.2)	82.7 (34.3)	.06
D-dimer (ng/mL)[c]	287 (200, 424)	659 (463, 2950)	<.001
Antithrombin activity (%)[c]	107 (10.08)	84.1 (12.4)	<.001

Abbreviations: A:G, albumin to globulin ratio; MA, maximum amplitude; R, reaction; TEG, thromboelastogram.
[a]Presented as the mean (SD) for normally distributed data and the median (interquartile range) for non-normal data.
[b]Based on Student *t* tests for normally distributed data and Mann-Whitney *U* tests for non-normal data.
[c]Significant difference between groups ($P < .05$).

aortic macrothrombus had no evidence of microthrombi. Two of the dogs diagnosed with macrothrombi, had hemostatic changes consistent with non-overt DIC,[16,18] while the third dog had hemostatic changes consistent with overt DIC. The sample size in this study was too small to draw any conclusions regarding the hematological and hemostatic variable abnormalities in tumor-bearing dogs with macrothrombi.

Hematological differences between healthy unaffected dogs and tumor-bearing dogs identified in this study were similar to previous studies and included mild anemia, neutrophilic leukocytosis and monocytosis.[54] Anemia in cancer patients is most commonly secondary to blood loss, red blood cell lysis, anemia of inflammatory disease and tumor-mediated cytokine release, resulting in iron sequestration and erythropoietin inhibition.[55,56] The neutrophilic leukocytosis and monocytosis was most likely secondary to inflammation, ulceration and necrosis associated with 81% of the tumor-bearing dogs, and tumor-mediated cytokine release.[57]

Inflammation and hemostasis are interdependent and inflammatory conditions might result in hypercoagulable states.[58,59]

TABLE 4 Comparison of hematological, acute phase proteins, and hemostatic variables between healthy unaffected dogs and tumor-bearing dogs with intracavitary hemorrhage diagnosed with carcinoma or sarcoma (excluding dogs with macrothrombi).

Variable	Healthy unaffected dogs[a]	Tumor-bearing dogs with intracavitary hemorrhage[a]	*P* value[b]
Hematology	n = 20	n = 10	
Hemoglobin (g/dL)[c]	17.7 (17.0, 18.5)	6.15 (4.90, 10.4)	<.001
Red blood cell count ($\times 10^6$/mm^3)[c]	7.82 (7.16, 8.49)	2.58 (2.29, 4.29)	<.001
Hematocrit (%)[c]	0.53 (0.51, 0.56)	0.20 (0.17, 0.31)	<.001
Mean corpuscular volume (fL)[c]	68.6 (66.2, 72.1)	73.3 (68.1, 77.4)	.01
Mean corpuscular hemoglobin concentration (g/dL)[c]	33.4 (32.8, 33.5)	32.5 (29.1, 33.1)	.04
Red cell distribution width (%)[c]	13.6 (13.1, 13.9)	17.4 (15.5, 19.4)	<.001
White cell count ($\times 10^3$/mm^3)[c]	8.27 (7.24, 9.80)	21.0 (12.7, 28.7)	<.001
Neutrophils ($\times 10^3$/mm^3)[c]	6.45 (1.84)	20.5 (11.2)	<.001
Band neutrophils ($\times 10^3$/mm^3)[c]	0 (0, 0)	0.37 (0, 0.45)	.003
Lymphocytes ($\times 10^3$/mm^3)[c]	1.34 (0.53)	0.78 (0.49)	.009
Monocytes ($\times 10^3$/mm^3)[c]	0.41 (0.30, 0.49)	1.13 (0.66, 1.90)	<.001
Eosinophil ($\times 10^3$/mm^3)[c]	0.38 (0.30, 0.55)	0.13 (0, 0.31)	.02
Basophil ($\times 10^3$/mm^3)	0 (0, 0)	0 (0, 0)	.34
Platelets ($\times 10^3$/mm^3)[c]	261 (222, 300)	131 (49, 190)	<.001
Acute phase proteins	n = 20	n = 10	
C-reactive protein (μg/mL)[c]	<10 (<10, <10)	46.2 (35.0, 64.0)	<.001
Serum amyloid A (μg/mL)[c]	<2 (<2, <2)	31.4 (20.6, 46.0)	<.001
Hemostasis	n = 20	n = 10	
TEG R time (min)	3.53 (1.15)	3.49 (1.64)	.94
TEG K (min)	1.8 (1.5, 2.0)	2.5 (1.2, 6.8)	.45
TEG angle (°)	65.2 (62.1, 69.2)	50.8 (38.2, 71.9)	.07
TEG MA (mm)	59.7 (58.0, 62.5)	48.7 (32.4, 60.0)	.07
TEG G (dyn/cm)	7.4 (6.9, 8.4)	4.75 (2.40, 7.50)	.06
TEG lysis 30 (%)	0 (0, 0)	0 (0, 0)	.91
TEG lysis 60 (%)	1.10 (0.30, 1.95)	0.15 (0, 0.60)	.13
	n = 20	n = 10	
Fibrinogen (mg/dL)[c]	324 (261, 368)	165 (72, 269)	.007
Factor X activity (%)[c]	100 (16.7)	48.3 (28.7)	<.001
Factor VII activity (%)[c]	100 (32.2)	56.3 (27.9)	.001
D-dimer (ng/mL)[c]	287 (200, 424)	5757 (3595, 6000)	<.001
Antithrombin activity (%)[c]	107 (10.08)	67.0 (19.5)	<.001

Abbreviations: A:G, albumin to globulin ratio; MA, maximum amplitude; R, reaction; TEG, thromboelastogram.
[a]Presented as the mean (SD) for normally distributed data and the median (interquartile range) for non-normal data.
[b]Based on Student *t* tests for normally distributed data and Mann-Whitney *U* tests for non-normal data.
[c]Significant difference between groups ($P < .05$).

Inflammatory cytokines, produced by immune cells in the tumor microenvironment as well as the tumor itself, are the main mediators of inflammation-induced activation of hemostasis.[60-62] White cell count, CRP and SAA, were significantly higher in the tumor-bearing dogs compared to healthy unaffected dogs. Inflammation, ulceration or necrosis of the tumors secondary to rapid tumor growth, insufficient blood supply and microthrombi, most likely contributed to the increases in CRP and SAA concentrations.[63,64] Inflammation and necrosis of the tumor was present in almost equal proportions of dogs with carcinoma or sarcoma (80% and 81%, respectively). This finding was in contrast to the recently reported retrospective study where sarcomas contributed 80% toward cases with necrosis.[10] The presence of inflammation and necrosis of a tumor was not substantially different in dogs with or without microthrombi (84% and 77%, respectively), despite this, band neutrophil, CRP and SAA concentrations were significantly higher in tumor-bearing dogs with microthrombi compared to dogs without microthrombi. This might be explained by a greater degree of inflammation and necrosis in the tumors with

TABLE 5 Comparison of hematological, acute phase protein, and hemostatic variables between tumor-bearing dogs with carcinoma or sarcoma (excluding 3 dogs with macrothrombi and 10 dogs with intracavitary hemorrhage).

Variable	Dogs with carcinoma[a]	Dogs with sarcoma[a]	*P* value[b]
Complete blood count	n = 28	n = 21	
Hemoglobin (g/dL)	12.8 (9.90, 15.2)	14.3 (9.90, 14.8)	.86
Red blood cell count ($\times 10^6$/mm^3)	5.50 (4.70, 6.77)	6.07 (4.60, 6.40)	.89
Hematocrit (%)	0.39 (0.31, 0.45)	0.43 (0.30, 0.44)	.88
Mean corpuscular volume (fL)	67.9 (66.8, 70.1)	67.6 (65.5, 70.2)	.59
Mean corpuscular hemoglobin concentration (g/dL)	32.3 (32.3, 33.8)	32.8 (32.6, 33.4)	.27
Red cell distribution width (%)	14.05 (13.3, 15.6)	13.7 (13.0, 15.2)	.40
White cell count ($\times 10^6$/mm^3)	17.0 (11.7, 21.9)	16.1 (11.6, 20.5)	.79
Neutrophils ($\times 10^6$/mm^3)	15.1 (8.99)	15.4 (9.63)	.91
Band neutrophils ($\times 10^6$/mm^3)	0 (0, 0.22)	0 (0, 0.26)	.89
Lymphocytes ($\times 10^6$/mm^3)	1.75 (1.02)	1.29 (0.47)	.07
Monocytes ($\times 10^6$/mm^3)	0.95 (0.65, 1.36)	0.99 (0.59, 1.40)	.96
Eosinophil ($\times 10^6$/mm^3)[c]	0.43 (0.19, 0.88)	0.20 (0, 0.36)	.02
Basophil ($\times 10^6$/mm^3)	0	0	.13
Platelets ($\times 10^6$/mm^3)	416 (282, 630)	355 (289, 471)	.41
Acute phase proteins	n = 28	n = 21	
C-reactive protein (μg/mL)	51.5 (32.4, 107)	97.5 (52.5, 197)	.12
Serum amyloid A (μg/mL)	22.7 (6.21, 101)	49.3 (11.4, 206)	.45
Hemostasis	n = 26	n = 19	
TEG R time (min)	3.86 (0.64)	3.51 (0.73)	.09
TEG K (min)	1.2 (0.9, 1.3)	1.1 (0.9, 1.4)	.87
TEG angle (°)	73.2 (70.2, 77.7)	74.5 (67.5, 77.8)	.94
TEG MA (mm)	76.1 (68.2, 80.1)	75.3 (64.6, 79.6)	.75
TEG G (dyn/cm)	16.0 (10.7, 20.1)	15.2 (9.1, 19.5)	.75
TEG lysis 30 (%)	0 (0, 0.4)	0.5 (0, 1.35)	.27
TEG lysis 60 (%)	0.50 (0.1, 2.5)	2.30 (0.85, 4.15)	.06
	n = 28	n = 19	
Fibrinogen (mg/dL)	822 (472, 903)	630 (488, 100)	.99
Factor X (%)	81.9 (26.0)	74.7 (17.4)	.30
Factor VII (%)	89.7 (33.7)	72.3 (33.3)	.09
D-dimer (ng/mL)	649 (366, 1999)	724 (521, 4965)	.21
Antithrombin (%)	85.4 (12.6)	82.0 (12.2)	.36

Abbreviations: A:G, albumin to globulin ratio; MA, maximum amplitude; n, number; TEG, thromboelastogram.
[a]Presented as the mean (SD) for normally distributed data and the median (interquartile range) for non-normal data.
[b]Based on Student *t* tests for normally distributed data and Mann Whitney *U* tests for non-normal data.
[c]Significant difference between groups ($P < .05$).

subsequent activation of hemostatic mechanisms and a greater predisposition to microthrombi formation. Alternatively, microthrombi might result in hypoxia, cell death, lysis, and subsequent inflammation within the tumor and in distant locations.

Increased platelet concentration identified in tumor-bearing dogs without hemorrhage in this study is most likely related to a combination of chronic inflammation, chronic consumption resulting in increased production,[65] and higher thrombopoietin production in dogs with carcinoma.[66] Cancer, particularly carcinoma, and inflammation were identified as the most and second most common causes of thrombocytosis in dogs.[67] In contrast, thrombocytopenia has been associated with hemangiosarcomas,[14] and DIC in tumor-bearing dogs.[18] In this study, the significantly decreased platelet concentration in dogs with hemorrhage is most likely from loss and consumption associated with bleeding and DIC as the majority of tumor-bearing dogs with hemorrhage had visceral hemangiosarcoma with concurrent active or recent hemorrhage. Additionally, platelet concentrations were significantly lower in tumor-bearing dogs with microthrombi compared to tumor-bearing

TABLE 6 Comparison of hematological, acute phase protein, and hemostatic variables in tumor-bearing dogs with and without microthrombi (excluding 3 dogs with macrothrombi and 10 dogs with intracavitary hemorrhage).

Variable	Dogs with microthrombi[a]	Dogs without microthrombi[a]	*P* value[b]
Complete blood count	n = 21	n = 28	
Hemoglobin (g/dL)	11.7 (9.30, 14.5)	13.3 (11.3, 15.7)	.11
Red blood cell count ($\times 10^6$/mm^3)	5.06 (4.06, 6.38)	5.83 (4.75, 6.88)	.19
Hematocrit (%)	0.34 (0.29, 0.43)	0.41 (0.34, 0.47)	.12
Mean corpuscular volume (fL)	68.2 (65.4, 70.1)	67.8 (66.8, 70.3)	.61
Mean corpuscular hemoglobin concentration (g/dL)	32.8 (32.1, 33.4)	33.2 (32.6, 33.8)	.31
Red cell distribution width (%)[c]	14.9 (13.5, 15.9)	13.5 (12.8, 14.6)	.01
White cell count ($\times 10^3$/mm^3)	16.7 (12.5, 21.6)	16.1 (10.6, 22.1)	.64
Neutrophils ($\times 10^3$/mm^3)	15.5 (9.59)	15.6 (9.02)	.77
Band neutrophils ($\times 10^3$/mm^3)[c]	0.12 (0, 0.56)	0 (0, 0)	.002
Lymphocytes ($\times 10^3$/mm^3)	1.57 (1.00)	1.54 (0.75)	.90
Monocytes ($\times 10^3$/mm^3)	0.99 (0.13, 0.58)	0.96 (0.60, 1.46)	.73
Eosinophil ($\times 10^3$/mm^3)	0.36 (0, 0.53)	0.21 (0.16, 0.62)	.72
Basophil ($\times 10^3$/mm^3)	0	0	N/A
Platelets ($\times 10^3$/mm^3)[c]	334 (233, 471)	416 (344, 605)	.03
Acute phase proteins	n = 21	n = 28	
C-reactive protein (μg/L)[c]	117 (52.6, 197)	52.0 (18.0, 87.7)	.01
Serum amyloid A (μg/L)[c]	77.5 (11.4, 429)	24.5 (2.87, 69.1)	.05
Hemostasis	n = 19	n = 25	
TEG R time (min)[c]	4.00 (0.55)	3.50 (0.73)	.02
TEG K (min)	1.2 (0.9, 1.4)	1.1 (0.8, 1.3)	.61
TEG angle (°)	73.9 (70.3, 76.8)	74.2 (68.7, 78.3)	.52
TEG MA (mm)	75.2 (70.5, 78.2)	75.9 (65.6, 80.6)	.55
TEG G (dyn/cm)	15.2 (12.1, 17.9)	15.7 (9.5, 20.8)	.55
TEG lysis 30 (%)	0.1 (0, 2.0)	0 (0, 0.6)	.28
TEG lysis 60 (%)	1.00 (0.5, 4.9)	1.0 (0.2, 2.9)	.36
	n = 20	n = 27	
Fibrinogen (g/dL)	835 (498, 992)	733 (487, 867)	.26
Factor X (%)	77.2 (23.8)	80.3 (22.2)	.66
Factor VII (%)	87.0 (38.7)	79.4 (31.0)	.46
D-dimer (ng/mL)[c]	1609 (591, 4063)	568 (338, 810)	.02
Antithrombin (%)	80.7 (11.7)	86.6 (12.6)	.11

Abbreviations: A:G, albumin to globulin ratio; MA, maximum amplitude; n, number; TEG, thromboelastogram.
[a]Presented as the mean (SD) for normally distributed data and the median (interquartile range) for non-normal data.
[b]Based on Student *t* tests for normally distributed data and Mann Whitney *U* tests for non-normal data.
[c]Significant difference between groups ($P < .05$).

dogs without microthrombi and is most likely associated with platelet consumption secondary to the persistent hypercoagulable state and subsequent microthrombi formation,[21,48] as evident in people with cancer and VTE,[68] and experimentally induced pulmonary microthromboemboli in dogs.[21-23]

Tumor-bearing dogs without hemorrhage had significantly lower TEG K time and higher angle, MA and G compared to healthy unaffected dogs in this study, indicative of a hypercoagulable state.[41] Thromboelastometry-based-hypercoagulability has previously been reported in 45% to 66% of dogs with various tumors,[11,13] 46% of dogs with carcinomas,[12] and 80% of dogs with *Spirocerca lupi*-induced sarcomas.[40] In dogs with DIC, hypercoagulability was the most common hemostatic abnormality when tissue-factor activated TEG was used to evaluate the hemostasis status.[42] This hypercoagulable state and associated inflammatory state is considered the early stage of DIC, now more commonly referred to as non-overt DIC, which carries a better prognosis than a hypocoagulable thromboelastogram seen in overt DIC.[18,42] Factors most likely contributing to the

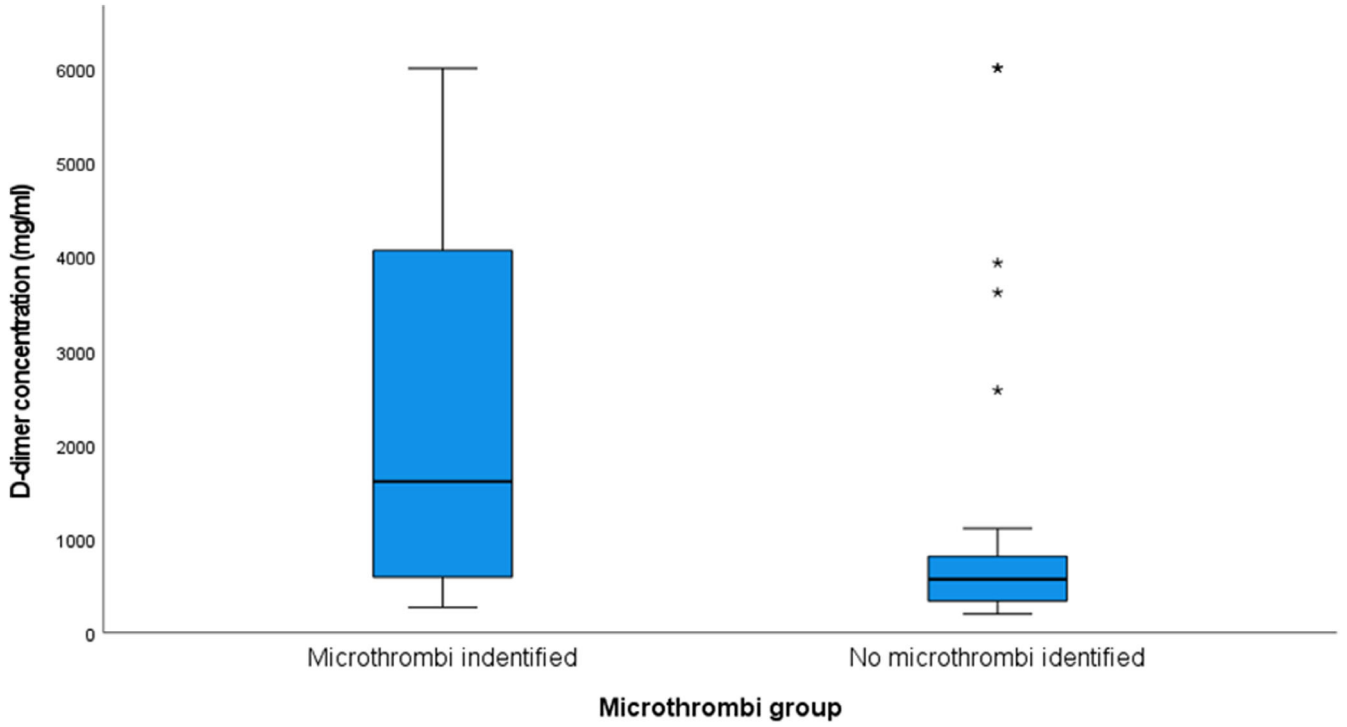

FIGURE 2 Boxplot of D-dimer concentration in dogs with carcinoma or sarcoma with (n = 21) or without (n = 28) microthrombi identified on post mortem (dogs with macrothrombi or intracavitary hemorrhage were excluded). D-dimer concentration was significantly increased in dogs with microthrombi compared to dogs without microthrombi ($P = .02$). Medians are represented by solid line within the boxplot. The whiskers denote the range extending to 1.5× the IQR from the upper and lower quartiles. * Represents 3rd quartile + 3× interquartile range.

hypercoagulable state in tumor-bearing dogs include tumor-released tissue factor and pro-coagulant microparticles; tortuous tumor neovascularization; inflammation, associated cytokines and fibrinogen driving hemostasis; increased platelet activity; and a lower hematocrit affecting particularly angle and MA.[69-74]

Fibrinogen concentration, an APP positively correlated to the severity of inflammation,[75] is most likely increased in the tumor-bearing dogs without hemorrhage due to the inflammation identified in these dogs resulting in the rate of fibrinogen synthesis exceeding its consumption.[76,77] Higher fibrinogen concentrations have previously been identified in dogs with various mammary gland tumors,[78,79] and in dogs with neoplastic transformation of *Spirocerca lupi* nodules.[40] The lower fibrinogen concentrations in the tumor-bearing dogs with hemorrhage is most likely a consequence of hemostatic consumption and intracavitary loss, consistent with overt DIC.[20]

The non-overt stage of DIC, evidenced by a low FX activity, hypercoagulable thromboelastogram, low AT activity and high D-dimer concentration was identified as the overall hemostatic state in dogs without intracavitary hemorrhage.[17,42,80] While overt DIC, evidenced by low FVII and FX activities, low platelet concentration, low AT activity and higher D-dimer concentration was identified as the overall hemostatic state in dogs with intracavitary hemorrhage. Using varying combinations of hemostatic changes, DIC has previously been reported in 12% of dogs with malignant solid tumors, and 50% of dogs with hemangiosarcoma.[14,15] The combination of local intra-tumoral hemostasis disturbances and systemic hemostatic dysfunction, identified as DIC in the tumor-bearing dogs in this study, is most likely fuelled by inflammation and tumor-related procoagulant microparticles resulting in the hypercoagulable state, consumption of clotting factors and subsequent microthrombi development and fibrinolysis.[73,81] Hemorrhage from friable blood vessels or structural damage to the tumor rather than a true coagulopathy might have resulted in sufficient intracavitary hemorrhage and subsequent fibrinolysis to satisfy the criteria of overt DIC, thereby introducing unavoidable bias in the definition of overt DIC in this study.

The D-dimer concentration, a specific indicator of cross-linked fibrin formation and subsequent fibrinolysis, was significantly increased in tumor-bearing dogs, with or without hemorrhage, compared to healthy unaffected dogs, as well as in tumor-bearing dogs with microthrombi. D-dimer concentration is considered the primary marker of VTE in people due to its high sensitivity and negative predictive value.[32] However, further diagnostic tests are required to confirm the diagnosis of VTE in people due to the low specificity.[30] Increased D-dimer concentrations have been reported in dogs with various cancer types with thromboemboli, but also in hemoabdomen, hemoperitonium, liver disease, heart or renal failure and post-surgically, all of which decrease the specificity of D-dimer concentration for the diagnosis of thromboembolic disease.[33,47,48] Explanations previously proposed for an increased D-dimer concentration in dogs with cancer without intracavitary effusion included a coagulopathy or micro-hemorrhage from damaged vessels.[33,48] However, our study found increased D-dimer concentrations in dogs without intracavitary hemorrhage, and was likely due to lysis of cross-linked fibrin

TABLE 7 Sensitivity and specificity for specific cut-offs, derived from receiver operator characteristic curve analysis for D-dimers, band neutrophils, platelets, and thromboelastography reaction time (TEG R) for the prediction of the presence of microthrombi in dogs with carcinoma or sarcoma.

Cut-off	Sensitivity % (95% CI)	Specificity % (95% CI)
D-dimer (ng/mL)		
>500	80 (63-98)	41 (22-59)
>1000	50 (28-72)	78 (62-94)
>2000	50 (28-72)	82 (67-96)
>3000	40 (19-62)	85 (72-99)
Band neutrophils ($\times 10^3/mm^3$)		
>0.00	52 (31-74)	89 (78-100)
>0.30	43 (22-64)	89 (78-100)
>0.60	19 (2-36)	96 (90-100)
Platelets ($\times 10^3/mm^3$)		
<200	14 (0-29)	96 (90-100)
<300	48 (26-69)	86 (73-98)
<400	67 (47-87)	64 (47-82)
<500	76 (58-94)	39 (21-57)
TEG R (min)		
>3	95 (85-100)	27 (10-44)
>3.8	68 (48-89)	73 (56-90)
>4.4	5 (0-15%)	88 (76-100)

Abbreviation: 95% CI, 95% confidence interval.

secondary to microthrombi formation and non-overt DIC.[51,73] Markedly increased D-dimer concentrations in dogs without pathologically identified microthrombi might have been caused by the underestimation of the prevalence of microthrombi as, despite extensive tissue sampling, the entire tumor and organs could not be evaluated histologically.

The good sensitivity (81%) and poor specificity (41%) of D-dimer concentration identified in this study, consistent with previous reports in dogs for the prediction of macrothrombi (sensitivity: 87%-100%, specificity: 26%-70%),[33,35,48,82] indicates a D-dimer concentration <500 ng/mL might be a useful screening test for the exclusion of the presence of recently formed microthrombi in dogs with carcinoma or sarcoma. D-dimer concentrations <500 ng/mL were identified in 4 dogs with microthrombi, indicating a normal D-dimer concentration does not exclude the possibility of the presence of microthrombi. Although tissue-factor activated TEG is insensitive for fibrinolysis,[83] tissue-plasminogen-activated TEG is considered sensitive in dogs, with a high specificity in people for the detection of fibrinolysis,[84] and might be superior to D-dimer concentration for identification of hyperfibrinolysis and microthrombi.

Platelet- and band neutrophil concentrations, as well as R were identified in multivariable analysis as predictive for the presence of microthrombi in this study. Unfortunately, none appear to be clinically useful as the cut-offs for all variables, are within their respective customary reference interval,[11,85,86] and similar to that of the healthy unaffected dogs in this study. However, a combination of the identified predictors of microthrombi and increased D-dimer concentration in a dog diagnosed with a carcinoma or sarcoma without intracavitary hemorrhage, should alert the practitioner to the possible presence of microthrombi. The clinical relevance of microthrombi to the dog and its hemostatic state, their role in metastasis, as well as the need for thromboprophylaxis to prevent development of overt DIC or reduce metastasis remains to be investigated.

Limitations of this study included the relatively low number of dogs in each tumor group and further larger prospective studies are required to validate the findings of this study. Statistical analysis was performed using the significant primary tumor, and the contribution of the surprisingly high percentage of dogs with incidental secondary and tertiary primary tumors to microthrombi development in this study is unclear.[71,72,87] The objective of the study was not to evaluate individual dogs for the presence of DIC, but rather identify the overall hemostatic state in tumor-bearing dogs that might predispose to microthrombi formation. The creation of reference intervals from 20 healthy dogs is not encouraged, contains an uncertainty inherent to the sample size and the inclusion of the $CI_{90\%}$ of each reference interval, histogram, median, and minimum and maximum values would make interpretation cumbersome.[88] This study was not designed to investigate the effect of comorbidities or medication on the development of microthrombi, but does reflect the real-life clinical presentation of tumor-bearing dogs.

In conclusion, thromboembolic disease, specifically microthrombi, are common in dogs with primary carcinoma or sarcoma in this prospective study. Overall, inflammatory and subsequent hemostatic dysfunction of overt and non-overt DIC identified in this study likely contributed to the development of microthrombi. Lower platelet concentration and increased TEG R time and D-dimer concentration was associated with microthrombi in tumor-bearing dogs without intracavitary hemorrhage. D-dimer concentration above 500 ng/mL might be a reasonable screening tool for the presence of microthrombi in dogs with carcinoma or sarcoma without intracavitary hemorrhage. The presence of non-overt DIC and microthrombi in other cancer types, the need for and types of therapeutic interventions and the effect on metastasis requires further investigation.

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CONFLICT OF INTEREST DECLARATION

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

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Research Ethics Committee of the Faculty of Veterinary Science, University of Pretoria (REC 105-18) and Animal Ethics Committee of the University of Pretoria (V100-18).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

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SUPPORTING INFORMATION

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

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