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RESEARCH ARTICLE

Evaluation of the performance of a human D-dimer test in dogs with neoplasia

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ABSTRACT

The goal of this study was to evaluate the suitability of a commercially available D-dimer assay as a diagnostic tool for testing dogs. This assay is an immunoturbidimetric diagnostic test, capable of determining the D-dimer levels in human plasma by using 2B9 monoclonal antibody. Plasma samples of clinically healthy (n = 20) and tumour-bearing (n = 50) dogs were measured. The tumours were grouped on the basis of histological type and aggressiveness, and then the measured D-dimer concentrations of these groups were compared to those of the control group. The differences were analysed statistically. For benign tumours, we did not find alterations in the D-dimer levels. However, in the case of malignant tumours (lymphoma, sarcoma, and carcinoma) and in the presence of metastases, significantly elevated D-dimer levels were measured. The assay proved to be suitable for measuring the D-dimer levels in plasma samples of dogs. The calculated reference range for dogs was confirmed to be between 0.06 and 0.69 μg/mL fibrinogen equivalent unit.

KEYWORDS

D-dimer, diagnostic test, dogs, neoplasia, tumour

INTRODUCTION

D-dimer is a cross-linked fibrin degradation product, the level of which increases in the blood during secondary fibrinolysis. In human medicine, deep vein thrombosis and pulmonary embolism are usually excluded by the determination of D-dimer concentration and a negative test result referring to a strictly determined cut-off level (Olson et al., 2011). The increase in D-dimer levels is not a disease-specific condition and may result from multiple causes. Therefore, it is not possible to set up a precise diagnosis based exclusively on the increase of D-dimer levels.

A connection between malignancy and thrombosis has been observed, as thromboembolism is present in almost half of cancer patients (Falanga and Rickles, 1999). Tumour cells can cause thrombosis by activating the blood clotting cascade or inhibiting the anticoagulant properties of vascular endothelial cells, monocytes, macrophages and platelets.

Based on human clinical studies, Nagy et al. (2012) reported a correlation between tumour aggressiveness and increased levels of D-dimer and suggested its potential use as a prognostic marker. Different tumour types have been examined and a correlation was found between elevated D-dimer levels in patients diagnosed with lung (Gabazza et al., 1993; Altiay et al., 2007), breast (Blackwell et al., 2000; Dirix et al., 2002; Khan et al., 2007),

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musculoskeletal (Morii et al., 2008), prostate (Adamson et al., 1993; Nakashima et al., 1995; Khoury et al., 2010), cervical (Gadducci et al., 1993) and colon (Kilic et al., 2008) cancers. However, articles investigating D-dimer levels of dogs with cancer are sporadic in the veterinary literature (Andreasen et al., 2012; de la Fuente et al., 2014; Font et al., 2015; Kang et al., 2016).

Based on the limited research data, we assumed that increased D-dimer levels would occur in dogs with malignant tumours. In order to test this hypothesis, we collected plasma samples from both tumour-bearing and clinically healthy dogs and analysed them with a human D-dimer test. Another purpose of this work was to evaluate the suitability of this assay as a diagnostic tool for malignant tumours on canine plasma samples.

The significance of D-dimer level determination in dogs is primarily in the diagnosis of pulmonary embolism and disseminated intravascular coagulation (DIC), but its levels may also be elevated in other diseases like thromboembolic diseases, bleeding, kidney, heart and liver failure, postoperative conditions and different types of cancers (Stokol, 2003; Giannoulopoulos et al., 2017). Nelson and Andreasen (2003) examined healthy dogs and dogs with pulmonary embolism. They found D-dimer concentrations under 250 ng/mL, corresponding to 0.5 µg/mL fibrinogen equivalent unit (FEU) in the control group, but concentrations were 2,000 ng/mL (4 µg/mL FEU) for the dogs with pulmonary embolism. Tick et al. (2008) came to the conclusion that high D-dimer values have a significant correlation with pulmonary embolism. Examining the diagnostic significance of D-dimer concentration in dogs with pulmonary embolism Epstein et al. (2013) reported that the application of a 250 ng/mL (0.5 µg/mL FEU) cut-off value made it possible to exclude pulmonary embolism with confidence.

Another thromboembolic disease affecting dogs is DIC, a severe thromboembolic and haemorrhagic disorder, always in the setting of some other disease, such as neoplasia, infection, pancreatitis, myocardial infarction, or immune-mediated haemolytic anaemia (IMHA) (Stokol et al., 2000). The diagnosis of DIC is not straightforward, and a definitive diagnosis can only be set up based on the results of several clinical examinations and laboratory tests (Giannoulopoulos et al., 2017). Stokol et al. (2000) examined 20 dogs suffering from DIC and 30 healthy dogs, and measured D-dimer levels by latex agglutination and immunoturbidimetric tests. They found that the average value for D-dimer concentration was higher in dogs with DIC than in healthy dogs. Several additional studies provide information on D-dimer concentration in dogs suffering from DIC. Caldin et al. (2000), Griffin et al. (2003) and Machida et al. (2010) found that D-dimer was useful as a diagnostic marker of DIC.

Based on data available in the veterinary literature, in most cases the D-dimer values were measured with assays intended for the measurement of human plasma samples utilising anti-human D-dimer monoclonal antibodies (Griffin et al., 2003; Stokol, 2003; Nelson, 2005; Machida et al., 2010). According to the principles of the measurement, there are immunoturbidimetry tests, such as Tina-quant® D-dimer (Boehringer Mannheim) (Caldin et al., 2000; Stokol et al., 2000), latex agglutination tests, like Accuclot™ D-dimer (Sigma Chemical Co, St Louis, MO) (Stokol et al., 2000; Nelson et al., 2009), and the NycoCard® point-of-care test based on an immunometric flow-through principle (Axis-Sheild PoC AS™) (Dewhurst et al., 2008).

Such tests were validated for use in the canine species (Giannoulopoulos et al., 2017), by applying a cut-off value adjusted for dogs. Griffin et al. (2003) examined dogs using a qualitative rapid immunochromatography diagnostic assay especially designed for use in the determination of D-dimer levels in canines; however, this test is no longer available commercially (Giannoulopoulos et al., 2017). To the best of our knowledge, no commercially available D-dimer diagnostic test specified for the analysis of canine plasma samples exists.

As mentioned above, the objective of the current work was twofold: (1) to evaluate the suitability of a commercially available human D-dimer test for D-dimer concentration measurement in dog plasma, and (2) to determine D-dimer concentration in plasma samples of dogs living with cancer and to assess the correlation between the presence of the tumour, the tumour type based on histology, tumour aggressiveness and D-dimer levels.

MATERIALS AND METHODS

Collection and handling of samples

We collected 70 plasma samples from dogs with the aid of the Veterinary Haematology and Oncology Centre and the Animal Health Centre of Budafok (Budapest, Hungary). The blood samples were placed in VACUETTE® (Greiner AG, Kremsmünster, Austria) phlebotomy tubes containing sodium citrate buffer, then the plasma was retrieved by centrifugation (2,000×*g*, 10 min).

Study population

The mean age of the dogs was 8 years and 2 months (1.5–14 years). The sex distribution was 52% males and 48% females. The examined animals represented 22 dog breeds, and 20 dogs were of mixed breed. Fifty samples were collected from dogs diagnosed with different types of tumours. The above-described population had the following classification based on the histological tumour types: mast cell tumour ($n = 6$), lymphoma ($n = 5$), sarcoma ($n = 10$), carcinoma ($n = 17$), insulinoma ($n = 3$), lipoma ($n = 3$), adenoma ($n = 3$), and other tumour types ($n = 3$), where tumours could not be classified into any of the previous groups.

The population of the tumour-bearing dogs is presented in Table A1. The control group consisted of 20 dogs judged to be clinically healthy. They were regularly vaccinated, treated with antiparasitic medicines and checked by a veterinarian. Animals that had no apparent illness or abnormalities detectable on cursory examinations were considered clinically healthy (Weiser and Allison, 2012).

All dogs underwent a clinical examination for complete systemic evaluation, health or diagnosis of tumour types,

and classification of body condition. Tumour diagnosis was made with the help of surgical interventions aimed at incisional or excisional biopsy and histopathological (immunohistopathological) examinations. Preoperative clinical evaluation included peripheral blood sampling for complete blood count, routine serum biochemical analyses for ionogram, total protein, albumin, liver function and kidney function. All dogs were clinically tested for vascular or bleeding disorders by platelet indices, buccal mucosal bleeding time tests and clinical diagnostic imaging techniques. Thoracic radiological (laterolateral right, laterolateral left and ventral-dorsal), and abdominal ultrasound examinations were performed to assess the presence of metastases. In some cases, computed tomography and magnetic resonance imaging examinations were also performed. Clinical stage classification was performed on the basis of tumour size (T), involvement of regional lymph nodes (N), and presence or absence of distant metastases (M), based on the TNM system (Owen, 1980). The macroscopic evaluation of regional lymph nodes was performed by palpation. If even a slight enlargement was observed, neoplastic involvement was confirmed by histopathological examination of excised lymph nodes.

Quantitative determination of D-dimer level

The canine plasma samples were tested by the Dia-D-Dimer immunoturbidimetric diagnostic assay (Diagon Ltd., Budapest, Hungary) that has been developed recently, based on a newly identified D-dimer specific monoclonal antibody 2B9 (Török-Nagy et al., 2019a). The test was validated for the analysis of human plasma samples by comparison to commercially available tests (Török-Nagy et al., 2019b). D-dimer concentrations were determined by turbidity measurements, performed on a Coag XL coagulometer (Diagon Ltd.).

Statistical analysis of the results

The reference range was set up on the basis of the measurement results of samples from dogs judged to be healthy. This group had normal distribution, as proved by the Shapiro–Wilk test. The P value was 0.1071 and the W value was 0.9217, which fall into the 95% acceptance range. Tumour-positive and tumour-negative groups were separated by the measured data, based on that reference range.

The D-dimer concentration results were displayed by box plots for tumour-bearing and healthy dogs (Tukey, 1977).

The differences between certain groups (tumour-bearing dogs, and certain tumour types compared to the control group) were analysed by non-parametric Wilcoxon–Mann–Whitney test. $P < 0.05$ was considered to be a significant difference. Statistical calculations were carried out using Minitab 14.0 statistical software (Minitab Inc., State College, PA).

An attempt was made to determine the sensitivity and specificity of the Dia-D-Dimer test for tumour-bearing dogs. For this purpose, determination of tumour types was carried out according to their morphology, histology and immunohistochemistry (IHC) test results.

RESULTS

The distribution of D-dimer concentrations measured in the samples of tumour-bearing dogs (benign, malignant, and metastatic tumours) and in the control dog groups is shown in Table 1.

The median of D-dimer concentrations in the control and tumour-bearing groups and the concentration ranges are shown in Table 2. Based on the diagnosis, the different neoplasm types were divided into two groups and classified as malignant or benign tumours, where this classification could clearly be done. The D-dimer results of the benign tumour group did not differ significantly from those of the control group (median = 0.34 μg/mL FEU; $P = 0.1310$). However, the concentration values of the malignant group displayed significant differences from those of the control group (median = 0.68 μg/mL FEU; $P = 0.0002$). Within the control group, D-dimer concentration values were low and fell in the range of 0.10–0.58 μg/mL FEU, while in the tumour-bearing group the range was much wider, including the highest value of 28.20 μg/mL FEU.

The results of animals with metastatic cancer were examined separately as shown in Table 3. The median of D-dimer levels in samples of dogs with metastatic cancer was 1.01 μg/mL FEU, higher than that of the overall malignant tumour group. Similarly, a statistically significant difference was recorded between the metastatic and the control group ($P = 0.0016$).

The plasma samples were grouped also on the basis of the histological grades and stages of tumours where this information was available. The D-dimer concentration ranges and medians are indicated in Table 4. Regarding the grades, in case of grade I the median was 0.40 μg/mL FEU

Table 1. Distribution of D-dimer concentrations measured in the samples of tumour-bearing and control dogs

Group (n)	D-dimer (μg/mL FEU)				
	0.1–0.5	0.5–1	1–2	2–3	>3
Control ($n = 20$)	18	2	–	–	–
Benign tumour ($n = 8$)	6	2	–	–	–
Malignant tumour ($n = 36$)	17	6	6	2	5
Metastasis ($n = 6$)	2	1	1	–	2

FEU = fibrinogen equivalent unit.

Table 2. D-dimer concentration ranges measured in the control and the tumour-bearing dog groups, calculated medians and P values for the evaluation of difference from the control group

Group (n)		D-dimer (μg/mL FEU)		
		Range	Median	P value
Control ($n = 20$)		0.10–0.58	0.25	–
Neoplasia	Benign ($n = 8$)	0.10–0.72	0.34	0.1310
	Malignant ($n = 42$)	0.10–28.20	0.68	0.0002

Table 3. D-dimer concentration ranges, calculated medians of the tumour-bearing animals and *P* values for the evaluation of difference from the control group

Group (*n*)	D-dimer (μg/mL FEU)		
	Range	Median	*P* value
Benign tumour (*n* = 8)	0.10–0.72	0.34	0.1310
Malignant tumour (*n* = 36)	0.10–15.50	0.57	0.0007
Metastasis (*n* = 6)	0.25–28.20	1.01	0.0016

Table 4. D-dimer concentration ranges and medians in the case of tumours of different histological grades (A) and stages (B)

A Grade (*n*)	D-dimer (μg/mL FEU)	
	Range	Median
I (*n* = 22)	0.10–15.50	0.40
II–III (*n* = 28)	0.10–4.75	0.94

B Stages (*n*)	D-dimer (μg/mL FEU)	
	Range	Median
I–II–III (*n* = 40)	0.10–4.74	0.44
IV–V (*n* = 10)	0.19–28.20	1.50

while for grades II–III, a higher median (0.94 μg/mL FEU) was found. We also found that the medians increased in the case of higher stages (1.50 μg/mL FEU).

We obtained outstandingly high D-dimer concentrations in the case of three samples. One of them was the sample of an 8-year-old male Rottweiler with a value of 15.50 μg/mL FEU. This dog was diagnosed with biliary carcinoma. Another sample originated from a spayed female mongrel with mammary carcinoma with metastases in the thorax (15.10 μg/mL FEU). The highest concentration value of 28.20 μg/mL FEU was measured in the sample of an 8-year-old, male Dogo Argentino with advanced-stage T-cell lymphoma.

The tumour-bearing groups were additionally examined on the basis of tumour type. The distribution of the measured D-dimer concentrations according to the different tumour types is shown in Table 5. Concentration ranges, calculated medians and *P* values showing the difference of the groups from the control group are presented in Table 6.

The highest D-dimer median was calculated in the group of dogs with malignant lymphoma (1.72 μg/mL FEU). A significantly high concentration value of 0.83 μg/mL FEU was also acquired from the animal group suffering from carcinoma. In the group with lipoma and insulinoma, the medians were low, similar to those of the control group. It should be added that the sample number was low in these groups. According to the results of the Mann–Whitney statistical test, the mast cell tumour group ($P = 0.1024$) did not differ significantly from the control group. In contrast, the lymphoma ($P = 0.0114$), sarcoma ($P = 0.0005$) and carcinoma ($P = 0.0022$) groups displayed significant differences (Table 6).

Table 5. D-dimer concentration distributions in groups with certain tumour types and in the control group

Group (*n*)	D-dimer (μg/mL FEU)				
	0.1–0.5	0.5–1	1–2	2–3	>3
Mast cell tumour (*n* = 6)	4	–	–	2	–
Lymphoma (*n* = 5)	1	1	1	–	2
Sarcoma (*n* = 10)	4	4	1	–	1
Carcinoma (*n* = 17)	7	2	5	–	3
Lipoma (*n* = 3)	2	1	–	–	–
Adenoma (*n* = 3)	2	1	–	–	–
Insulinoma (*n* = 3)	2	–	–	–	1
Other tumours (*n* = 3)	3	–	–	–	–
Control (*n* = 20)	18	2	–	–	–

Table 6. D-dimer concentration ranges and medians in groups with certain tumour types and in the control group

Tissue type	Group (*n*)	Range Median D-dimer (μg/mL FEU)		*P* value
	Control (*n* = 20)	0.1–0.58	0.25	–
Connective	Lipoma (*n* = 3)	0.26–0.51	0.31	NA
	Mast cell tumour (*n* = 6)	0.10–2.38	0.38	0.1024
	Sarcoma (*n* = 10)	0.12–3.77	0.67	0.0005
Epithelial	Adenoma (*n* = 3)	0.10–0.72	0.48	NA
	Carcinoma (*n* = 17)	0.10–15.5	0.83	0.0022
Lymphatic system	Lymphoma (*n* = 5)	0.14–28.2	1.72	0.0114
Exocrine	Insulinoma (*n* = 3)	0.10–3.85	0.25	NA
–	Other tumours (*n* = 3)	0.10–0.37	0.26	NA

NA: Not applicable due to lack of type or low statistical power.

The correlations between certain groups and the control group are presented as a box plots in Figs. 1 and 2.

Based on the values measured in healthy dogs, we propose to set up a reference range of 0.06–0.69 μg/mL FEU that can be used for the analysis of plasma samples from dogs with neoplasms.

Positive and negative groups were created from the measured data based on this reference range. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) of the Dia-D-Dimer test were also calculated with altered cut-off values, based on the diagnosis of the dogs (healthy and not healthy categories). According to our results, with cut-off values extending to 0.30–0.69 μg/mL FEU, sensitivity was between 33 and 51%, while specificity was between 85 and 100% (Table 7). By the application of the

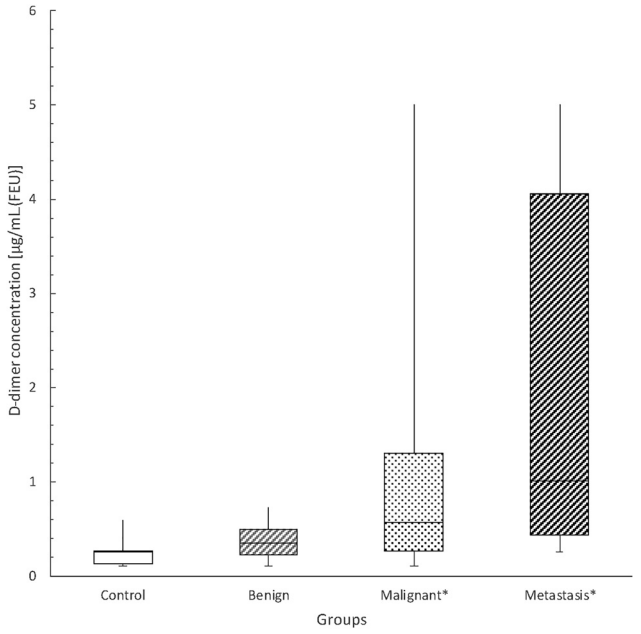

Fig. 1. Box plot display of the D-dimer concentrations of the control group and of dogs with benign, malignant, and metastatic tumours. Asterisks indicate significant difference from the control group

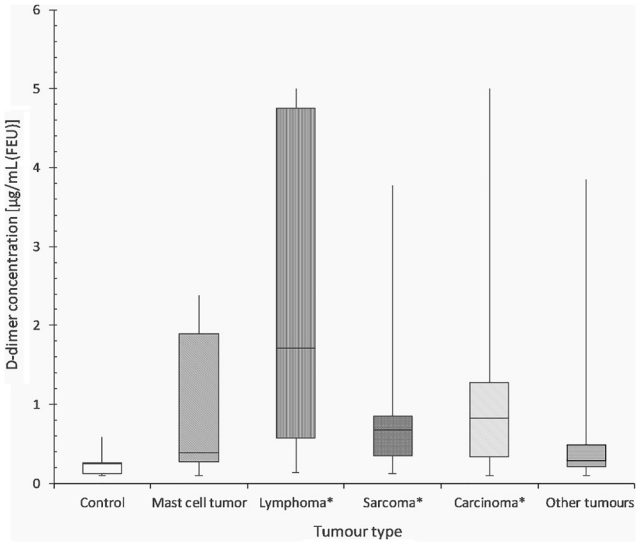

Fig. 2. Box plot display of the D-dimer concentrations of the control group and of certain tumour type groups. Asterisks indicate significant difference from the control group

upper limit of the reference range (0.69 µg/mL FEU), with 33% sensitivity, we reached 100% specificity. The PPV increased to 100% and the NPV was 29% for samples of tumour-bearing dogs.

Therefore, when using the Dia-D-Dimer test, samples with a concentration lower than the cut-off value of 0.69 µg/mL FEU should be regarded as negative in respect of malignancy, while samples with results above that value should be considered likely to come from a malignant tumour case.

Table 7. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) values of the Dia-D-Dimer test for tumours, expressed in percentages, with the application of different cut-off values

D-dimer cut-off (µg/mL FEU)	Sensitivity %	Specificity %	PPV %	NPV %
>0.3	51	85	92.5	32.1
>0.4	41	85	90.9	28.3
>0.5	37	90	93.1	28.1
>0.6	33	100	100	29
>0.69	33	100	100	29

DISCUSSION

The results of our examinations supported the findings of the publications mentioned above, i.e. that the anti-human D-dimer specific monoclonal antibody reacts with the D-dimer in canine samples and that the Dia-D-Dimer test can be applied for the analysis of canine samples.

Although increased D-dimer levels may suggest thromboembolism, patients with tumour, inflammatory diseases and surgery may also provide positive results (Stokol, 2003; Morii et al., 2008). However, in veterinary medicine, the D-dimer assay is mostly used for the diagnosis of pulmonary embolism and DIC.

Our results showed that the concentrations measured in the tumour group were significantly higher than those found in the control group. These results are in line with other reports from the literature, mainly based on the analysis of human samples (Adamson et al., 1993; Gabazza et al., 1993; Gadducci et al., 1993; Nakashima et al., 1995; Blackwell et al., 2000; Dirix et al., 2002; Altiay et al., 2007; Khan et al., 2007; Kilic et al., 2008; Morii et al., 2008; Khoury et al., 2010; Nagy et al., 2012).

Nelson and Andreasen (2003) examined a group of dogs with neoplasia, where 7 out of 16 dogs were found to have increased D-dimer levels. The neoplastic group represented a borderline difference from the control group ($P = 0.06$). After having analysed multiple disease groups simultaneously, Dewhurst et al. (2008) reported that dogs with neoplasia had the highest D-dimer concentration (1.7 mg/L = 3.4 µg/mL FEU), a value that is similar to our results, supporting a correlation between increased D-dimer levels and neoplasia.

In the groups of dogs suffering from malignant lymphoma, sarcoma, and carcinoma, the D-dimer levels were found to be significantly higher than in the control group.

We did not find a significant difference between the values of dogs with malignant mast cell tumour and those of the control group. Moreover, in the group of dogs with benign lipoma and insulinoma, the median of the D-dimer concentration was almost the same as in the control groups.

In a Pakistani study, D-dimer and platelet concentrations were examined in healthy dogs and in dogs with a number of different diseases. Six disease groups were examined, including a group of dogs suffering from neoplasia ($n = 28$). It was shown that the D-dimer concentration was significantly increased in this cohort (Kang et al., 2016).

Andreasen et al. (2012) examined coagulation and fibrinolysis parameters in 71 dogs with malignant tumours and compared the changes in haemostatic parameters with the type of the tumour and progression of the disease. No differences were found in the D-dimer values between the animal groups suffering from different tumours (carcinoma, sarcoma, mast cell tumour, and lymphoma). The study was also extended to monitoring the progression of tumours, and dogs with distant metastatic disease had significantly higher fibrinogen and D-dimer values than dogs with locally invasive and locally non-invasive tumours (Andreasen et al., 2012).

We also found that the presence of metastases and the aggressiveness of the tumours are reflected in the D-dimer results. As compared to the control group, the analysis of the plasma samples of dogs in the malignant and metastatic tumour groups displayed significantly higher D-dimer concentrations. Three outstandingly high D-dimer levels were measured, as mentioned above. One of them was a dog with mammary carcinoma with metastasis in the thorax (Stage 5), while the other dog had T-cell lymphoma (Stage 4). The third dog was diagnosed with biliary carcinoma without information about metastases.

Morii et al. (2008) came to a similar conclusion when examining the samples of 77 human patients with tumour. According to their results, the D-dimer values were significantly higher for malignant tumours than for benign tumours.

Recent studies have revealed that plasma D-dimer levels are correlated with the stage and grade of the tumour (Kwon et al., 2008; Batschauer et al., 2010; Morii et al., 2011). Our results support the findings reported in the above-mentioned publications, thus confirming the correlation between higher tumour grade or stage and the increased D-dimer value.

It should be noted that in the veterinary literature on canine cancers, we did not find any information on the sensitivity and specificity of D-dimer level measurements. The data presented in Table 7 show that the alteration of the cut-off value significantly changes the sensitivity and specificity of the assay. In our examinations, the determined cut-off value (<0.69 μg/mL FEU) was higher than the cut-off value recommended by the manufacturer for the exclusion of deep vein thrombosis or pulmonary embolism in humans (<0.5 μg/mL FEU). With this cut-off value the test reached a high specificity and PPV, thus approximately 100% of dogs with a negative test result were clinically healthy. However, due to the low sensitivity of the test, only 33% of dogs diagnosed with cancer showed a positive test result.

Our results suggest that the examined Dia-D-Dimer diagnostic test is suitable for determining D-dimer levels in canine samples. However, further research is required to determine the magnitude of the diagnostic value of increased D-dimer levels in the prognosis of tumours in dogs and other animal species.

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APPENDIX

Table A1. Description of the population of the tumour-bearing dogs examined

Tumour type	Age (years)	Breed	Gender	Stage	Grade	Localisation		Metastasis	Histopathology
Mast cell tumour	1.5	Pinscher	Male	1	1	Skin	Leg	No	
Mast cell tumour	14	Fox Terrier	Male	1	2	Skin	Leg	No	
Mast cell tumour	13	Mongrel	Male	1	2	Skin	Trunk (flank)	No	
Mast cell tumour	5.5	Mongrel	Female	3	2	Skin	Head	No	
Mast cell tumour	3.5	Boxer	Female	1	2	Skin	Neck	No	
Mast cell tumour	6	American Staffordshire Terrier	Male	3	2	Skin	Trunk (chest)	No	
Lymphoma	11	Beagle	Female	1	1	Skin	Neck and back	No	Epithelial T-cell lymphoma
Lymphoma	7	Mongrel	Female	2	1	Lymph node	Multicentric	No	Indolent T-cell lymphoma
Lymphoma	10	Mongrel	Male	5	2	Mediastinum		No	Mediastinal T-cell lymphoma
Lymphoma	13	Mongrel	Male	5	3	Spleen		No	B-cell lymphoma
Lymphoma	8	Dogo Argentino	Male	4	2	Lymph node	Multicentric	Yes	Peripheral T-cell lymphoma
Sarcoma	7	Irish Setter	Male	1	1	Spleen		No	Fibrosarcoma
Sarcoma	6.5	German Shepherd	Male	2	1	Nasal cavity		No	Chondrosarcoma
Sarcoma	9	French Bulldog	Female	2	2	Front leg	Metacarpal area	No	Fibrosarcoma
Sarcoma	9	Boxer	Male	3	3	Front leg	Distal radius	No	Osteosarcoma
Sarcoma	10	Akita	Female	3	2	Hind limb	Proximal tibia	No	Osteosarcoma
Sarcoma	8	Mongrel	Male	1	1	Thigh		No	Peripheral nerve sheath tumour
Sarcoma	11	Mongrel	Male	1	1	Hind limb	Distal femur	No	Fibrosarcoma
Sarcoma	12	English Setter	Male	1	3	Oral cavity		No	Fibrosarcoma
Sarcoma	10	Pit Bull terrier	Female	1	1	Skin	Chest	No	Fibrosarcoma
Sarcoma	11	Bernese Mountain Dog	Female	1	1	Skin	Neck	No	Fibrosarcoma
Carcinoma	12	Fox Terrier	Female	4	3	Thyroid gland		Yes	Adenocarcinoma
Carcinoma	8	Puli	Female	4	2	Mammary gland		Yes	Tubulopapillary simplex carcinoma
Carcinoma	8	Doberman Pinscher	Male	3	3	Oral cavity		Yes	Squamous cell carcinoma
Carcinoma	6	Mongrel	Female	5	3	Lung		Yes	Tubulopapillary carcinoma
Carcinoma	10	Miniature Poodle	Female	2	2	Mammary gland		No	Solid carcinoma
Carcinoma	8	Labrador Retriever	Male	2	2	Urine bladder		No	Transitional cell carcinoma
Carcinoma	11.5	Mongrel	Male	3	2	Prostate		No	Prostate carcinoma
Carcinoma	8	Puli	Male	4	2	Oral cavity		No	Squamous cell carcinoma

(continued)

Table A1. **Continued**

Tumour type	Age (years)	Breed	Gender	Stage	Grade	Localisation		Metastasis	Histopathology
Carcinoma	10	Mongrel	Male	2	2	Lung		No	Small cell carcinoma
Carcinoma	7.5	Mongrel	Female	2	2	Skin	Chin	No	Squamous cell carcinoma
Carcinoma	13	Mongrel	Male	3	2	Liver		No	Biliary carcinoma
Carcinoma	10	French Bulldog	Male	4	2	Prostate		No	Prostate carcinoma
Carcinoma	8	Rottweiler	Male	4	1	Liver		No	Biliary carcinoma
Carcinoma	9	Yorkshire Terrier	Female	2	1	Mammary gland		No	Tubulopapillary simplex carcinoma
Carcinoma	6	Golden Retriever	Female	3	3	Nasal cavity		No	Anaplastic carcinoma
Carcinoma	11	Bichon Havanese	Male	4	3	Prostate		No	Prostate carcinoma
Carcinoma	7	French Bulldog	Female	1	1	Mammary gland		No	Tubulopapillary, complex carcinoma
Leukaemia	4.5	Staffordshire Terrier	Male	2	2	Blood		No	Myelogenous leukaemia, acute AML5a
Insulinoma	8	Mongrel	Male	2	1	Pancreas		No	Insulinoma
Insulinoma	9	Mongrel	Male	2	1	Pancreas		No	Insulinoma
Insulinoma	12	Mongrel	Female	3	2	Pancreas		Yes	Insulinoma
Lipoma	9	Labrador Retriever	Female	1	1	Skin	Axillar area	No	
Lipoma	12	Bichon Havanese	Female	1	1	Skin	Sternal area	No	
Lipoma	10.5	Mongrel	Female	1	1	Skin	Axillar area	No	
Adenoma	10	Mongrel	Female	1	1	Mammary gland		No	Papillary adenoma
Adenoma	12	Mongrel	Male	1	1	Perianal area		No	Perianal gland adenoma
Adenoma	9.5	Yorkshire Terrier	Female	1	1	Mammary gland		No	Intraductal papillary adenoma
Benign mixed tumour	9	Yorkshire Terrier	Female	1	1	Mammary gland		No	Benign mixed tumour
Papilloma	10.5	West Highland White Terrier	Male	1	1	Mammary gland		No	Papilloma