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NOTE

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Microparticle-associated tissue factor activity in dogs with disseminated intravascular coagulation

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ABSTRACT. Microparticle (MP)-associated tissue factor (TF) activity in plasma might play a role in human disseminated intravascular coagulation (DIC). The aim of this study was to compare MP-TF activity between non-DIC and DIC groups. Ten clinically healthy beagles and 26 diseased dogs were enrolled. The proportion of dogs with increased MP-TF activity was significantly higher in the DIC group than the non-DIC group (P=0.014). MP-TF activity in the DIC group was significantly higher than the non-DIC group (P=0.021). MP-TF activity positively correlated with plasma D-dimer concentration (r=0.42, P=0.034). Moreover, MP-TF activity was decreased by the time of recovery in some dogs with DIC. Larger prospective studies are warranted to assess its value as a diagnostic and prognostic biomarker in DIC.

KEY WORDS: disseminated intravascular coagulation, dog, microparticle, procoagulant activity, tissue factor

Tissue factor (TF) is a transmembrane protein that functions as the principal initiator of the extrinsic coagulation cascade [12]. Therefore, TF is considered to play a central role in disseminated intravascular coagulation (DIC). Importantly, TF can be associated with circulating microparticles (MPs) in the blood. TF-bearing MPs (TF-MPs) are primarily released from activated monocytes, vascular endothelial cells, platelets, and TF-expressed tumor cells [14].

In people, several studies have reported that MP-associated TF (MP-TF) activity is associated with increased risk of thrombosis and DIC [3, 4, 10, 19]. On the contrary, there have been few studies of MP-TF activity in dogs with a spontaneous disease. A previous study reported that some dogs with immune-mediated hemolytic anemia (IMHA) have higher MP-TF activity than clinically normal dogs [7]. Recently, we demonstrated that some dogs with a malignant tumor, including hemangiosarcoma (HSA), malignant melanoma, and high-grade lymphoma, have higher MP-TF activity than clinically normal dogs [9]. However, to the best of our knowledge, there have been no studies of the association between MP-TF activity and DIC in dogs. Thus, the aim of this study was to compare MP-TF activity between non-DIC and DIC groups consisting of dogs with various underlying diseases. In some cases, MP-TF activity was assessed during the clinical course.

A retrospective study was conducted with dogs referred to the Yamaguchi University Animal Medical Center (YUAMEC) from January 2015 to May 2018. Informed written consent was obtained from all dog owners at admission. All procedures were approved by the institutional ethics committee for animal clinical tests at the Joint Faculty of Veterinary Medicine in Yamaguchi University (approval No. 007).

Ten clinically healthy beagles maintained at YUAMEC and 26 client-owned diseased dogs that underwent coagulation-fibrinolysis tests at presentation were included in this study. Client-owned diseased dogs were diagnosed as DIC if abnormalities in 3 or more of the six parameters consisting of platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, antithrombin III (AT III) activity, and D-dimer concentration was detected [5]. Dogs that did not have results for all of six coagulation-fibrinolysis parameters were excluded from this study. The dogs were also excluded if platelet aggregations on blood smears and hemolysis of plasma samples were observed because they might be due to artificial errors in

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blood sampling, and hemolysis might increase MP-TF activity [7]. The dogs were also excluded if chyle of plasma samples was observed because lipid precipitates were contaminated into isolated MPs. A total of 26 cases fulfilled the inclusion criteria.

Platelet poor plasma (PPP) samples were collected from each dog and the MP-TF activity was measured using the FXa generation assay. The detailed information is shown in the Supplementary Materials and Methods.

Difference of MP-TF activity between the non-DIC and DIC groups was analyzed using Mann–Whitney *U* test. Difference of the proportion of dogs with increased MP-TF activity between the non-DIC and DIC groups was analyzed using Fisher's exact test. Correlation between MP-TF activity and plasma D-dimer concentration was analyzed by Spearman's rank correlation coefficient. *P* value of <0.05 was considered statistically significant.

Twenty-six diseased dogs were divided into non-DIC (n=14) and DIC (n=12) groups. The signalment, diagnosis, and MP-TF activity of healthy beagles and 26 diseased dogs are shown in Table 1. The mean and median MP-TF activity in 10 healthy control beagles was 5.3 ± 3.0 AU (mean ± SD) and 5.0 AU (range, 0.0–10.0 AU), respectively. The reference value of MP-TF activity was defined as ≤11.3 AU (mean + 2SD). Ten of 26 dogs (38.5%) had MP-TF activity greater than the reference value [HSA: 2 dogs, lymphoma: 3 dogs, acute pancreatitis (AP): 2 dogs, IMHA: 1 dog, leptospirosis: 1 dog, spindle cell sarcoma: 1 dog]. The proportion of dogs with increased MP-TF activity was significantly higher in the DIC group (8/12 dogs, 66.7%) than the non-DIC group (2/14 dogs, 14.3%) (*P*=0.014). Median MP-TF activity was 5.0 AU (range, 1.9–37.2 AU) and 26.6 AU (range, 1.6–85.2 AU) in the non-DIC and DIC groups, respectively (Fig. 1). MP-TF activity in the DIC group was significantly higher than the non-DIC group (*P*=0.021). MP-TF activity was positively correlated with plasma D-dimer concentration (*r*=0.42, *P*=0.034) (Fig. 2).

Repeated samples were available from 3 dogs during treatment, including dogs with splenic HSA (case no. 16), AP (case no. 23), and IMHA (case no. 25). The detailed results of coagulation-fibrinolysis tests in these dogs are shown in Table 2. MP-TF activities in these dogs were followed-up during the clinical course (Fig. 3A). In case no. 16, MP-TF activity was decreased from 85.2 AU at presentation to 19.6 AU after splenectomy. In case no. 23, the dog was diagnosed with AP depending on the results of serum biochemistry, SNAP cPL test (IDEXX, Tokyo, Japan), and abdominal ultrasonography. The dog gradually recovered with intensive care, and improvements of coagulation-fibrinolysis parameters were observed on day 9. MP-TF activity decreased from 62.5 AU

Table 1. Signalment, diagnosis, and microparticles-associated tissue factor (MP-TF) activity of healthy and diseased dogs

Cases	Breed	Age	Gender	Diseases	MP-TF activity (AU)
Healthy controls (n=10)	Beagles	4–10y	M (n=5), F (n=5)	Clinically healthy	5.3 ± 3.0 (mean)
Non-DIC					
1	Miniature Dachshund	11y0m	F	ALL	2.8
2	Doberman	7y9m	FS	Lymphoma (high-grade, T cell, renal)	1.9
3	Chihuahua	6y2m	MC	Lymphoma (high-grade, B cell, gastrointestinal)	4.1
4	Shiba	10y11m	MC	Lymphoma (high-grade, B cell, multicentric)	5.6
5	Polish Lowland Sheepdog	3y10m	MC	Lymphoma (high-grade, B cell, gastrointestinal)	6.7
6	Miniature Dachshund	16y2m	FS	Lymphoma (high-grade, B cell, multicentric)	**37.2**
7	French Bulldog	8y3m	FS	MCT	1.9
8	Labrador Retriever	10y9m	F	Mammary gland carcinoma, Hyperadrenocorticism	3.7
9	Jack Russell Terrier	9y7m	MC	Thyroid medullary carcinoma, MCT	6.4
10	Chihuahua	10y3m	F	AP, DM, Bilateral adrenomegaly	**13.3**
11	Shiba	4y5m	F	IBD	9.3
12	Chihuahua	5y10m	M	IMHA	10.8
13	Chihuahua	11y6m	F	Pyometra	2.5
14	Miniature Dachshund	11y7m	F	Pyometra	4.4
DIC					
15	Miniature Dachshund	9y10m	FS	HSA (splenic)	**28.5**
16	Flat-coated Retriever	9y0m	FS	HSA (splenic)	**85.2**
17	Shiba	8y7m	MC	Lymphoma (high-grade, T cell, hepatosplenic)	4.3
18	Mixed	8y8m	MC	Lymphoma (high-grade, T cell, gastrointestinal)	5.0
19	Shiba	6y1m	M	Lymphoma (high-grade, T cell, gastrointestinal)	8.1
20	Shiba	2y2m	M	Lymphoma (high-grade, T cell, mediastinal)	**24.8**
21	Miniature Dachshund	10y6m	FS	Lymphoma (high-grade, B cell, splenic)	**30.3**
22	Chihuahua	12y0m	MC	Splenic spindle cell sarcoma	**16.9**
23	Papillon	7y7m	M	AP	**62.5**
24	Pekingese	9y10m	FS	IMHA	1.6
25	Pomeranian	6y5m	M	IMHA	**34.3**
26	French Bulldog	0y6m	M	Leptospirosis	**36.7**

F, female; FS, spayed female; M, male; MC, castrated male; ALL, acute lymphocytic leukemia; MCT, mast cell tumor; SCC, squamous cells carcinoma; AP, acute pancreatitis; DM, diabetes mellitus; IBD, inflammatory bowel disease; HSA, hemangiosarcoma; IMHA, immune-mediated hemolytic anemia. MP-TF activity above the reference range (≤11.3 AU) calculated by the mean ± 2SD for healthy controls are indicated by boldface.

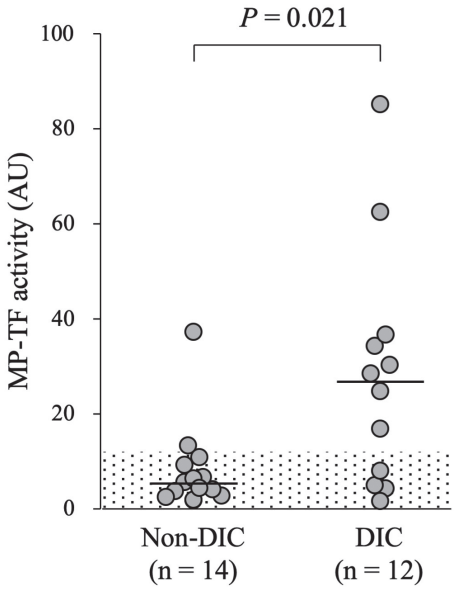

Fig. 1. Microparticle-associated tissue factor (MP-TF) activity in diseased dogs categorized into the non-disseminated intravascular coagulation (non-DIC) (n=14) and DIC (n=12) groups. The shaded region represents the reference range determined by the results measured in healthy controls (n=10). The line in each group indicated the median MP-TF activity value. *P* value is according to Mann–Whitney *U* test.

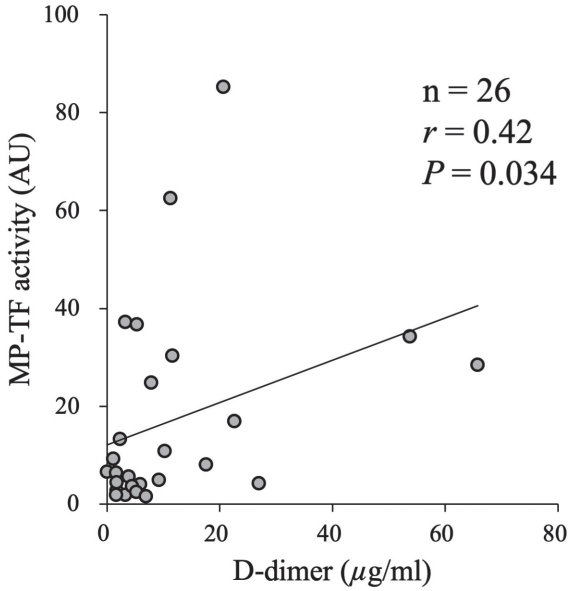

Fig. 2. Correlation between microparticle-associated tissue factor (MP-TF) activity and D-dimer concentration in 26 diseased dogs. Correlation between MP-TF activity and D-dimer concentration was analyzed by Spearman's rank correlation coefficient.

Table 2. Disease, parameters of coagulation and fibrinolysis, and desseminated intravascular coagulation (DIC) category of three dogs in follow-up study

Case no.	Disease	Day	PLT ($\times 10^4/\mu l$)	PT (sec)	aPTT (sec)	Fibrinogen (mg/dl)	AT III (%)	D-dimer (μg/ml)	DIC category
16	HSA	1	4.1	7.1	15.6	243.5	53.4	20.6	DIC
		21	63.2	7.1	9.5	127.6	81.0	0.3	non-DIC
23	AP	1	4.6	10.0	130.4	477.8	65.9	11.3	DIC
		9	17.2	5.9	11.9	158.7	119.3	3.8	DIC
25	IMHA	1	5.4	9.1	16.6	143.5	49.7	53.7	DIC
		14	8.7	6.6	9.3	352.5	91.0	4.9	non-DIC

Abnormal findings in coagulation-fibrinolysis tests are defined as follows: low platelet (PLT) count (<20 × $10^4/\mu l$), prolonged prothrombin time (PT) (>10.0 sec), prolonged activated partial thromboplastin time (aPTT) (>20.0 sec), low plasma fibrinogen concentration (<200 mg/dl), low plasma AT (antithrombin) III activity (<80%), and high plasma D-dimer concentration (>3.5 μg/ml). HSA, hemangiosarcoma; AP, acute pancreatitis; IMHA, immune-mediated hemolytic anemia.

at presentation to 5.1 AU on day 9. In case no. 25, the dog was diagnosed with primary IMHA depending on the findings of severe regenerative anemia, autoagglutination, and the other clinical examinations. The dog was gradually recovered with intensive care, and DIC cessation was confirmed on day 14. MP-TF activity was decreased from 34.3 AU at presentation to 6.2 AU on day 14. D-dimer concentrations also decreased in all the dogs when they were recovered (Fig. 3B).

In the present study, we found that some diseased dogs had increased MP-TF activity. We did not determine the cellular origin of circulating TF-MPs in these dogs. Recent studies demonstrated that TF expression and MP-TF activity were detected in some canine HSA cell lines but not lymphoma cell lines [9, 20]. Although TF expression in canine HSA *in situ* was not clarified, tumor cells-derived TF-MPs might directly contribute to the MP-TF activity in dogs with HSA. On the contrary, several studies of human patients with lymphoma suggest that hypercoagulability in patients with lymphoma is likely not secondary to tumor-derived TF [2, 18]. The hypothesis suggested in human lymphoma also seems plausible in dogs with lymphoma.

IMHA is an important cause of DIC and thrombosis in dogs [1]. TF mRNA expression is increased, and the concentration of cytokines associated with monocyte activation is elevated in blood from dogs with IMHA [8, 16]. Moreover, recent studies demonstrated that the number of TF-positive thrombocytes and MP-TF activity were increased in dogs with IMHA [6, 7]. Thus, increased MP-TF activity might be associated with thrombosis and DIC development in dogs with IMHA. In this study, MP-TF

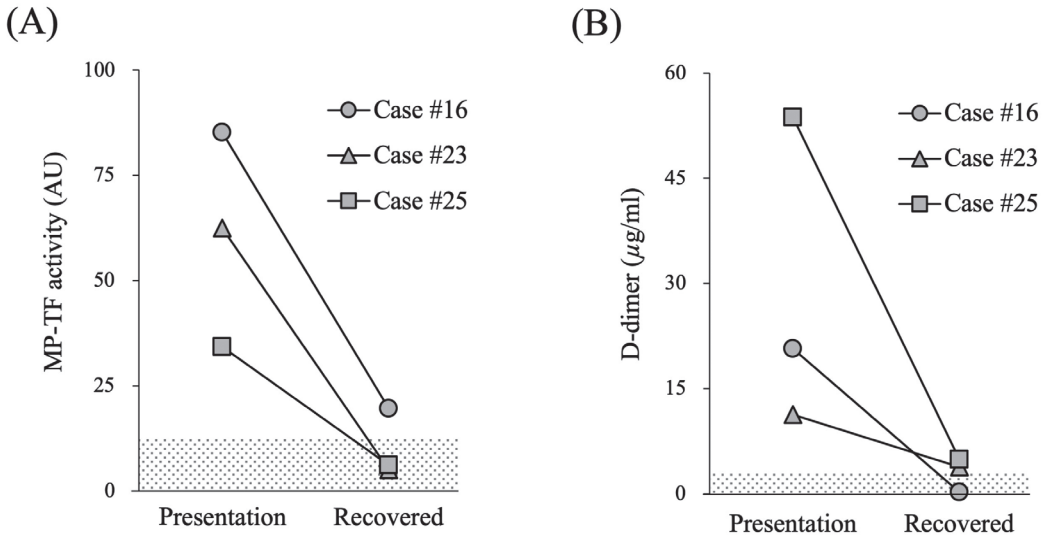

Fig. 3. Follow-up of three dogs with increased microparticle-associated tissue factor (MP-TF) activity. (A) MP-TF activity at presentation and the time of recovery in dogs with splenic hemangiosarcoma (cases no. 16), acute pancreatitis (case no. 23), and immune-mediated hemolytic anemia (case no.25). (B) Plasma D-dimer concentrations at presentation and the time of recovery in three dogs. The shaded region represents the reference range of each parameter.

activity was increased in a dog (case no. 25) but not in the other dog (case no. 24) among dogs with DIC secondary to IMHA. A previous report indicates that procoagulant activity with phosphatidylserine (PS)-positive MPs is increased in some dogs with IMHA in the absence of MP-TF activity [7]. Therefore, PS-MPs might also contribute to DIC development in dogs with IMHA.

In the present study, MP-TF activity in the DIC group was significantly higher than the non-DIC group. MP-TF activity was also positively correlated with D-dimer concentration. These results suggest that increased MP-TF activity reflects hypercoagulability and is associated with DIC development in dogs with various diseases. However, MP-TF activity may have increased as a consequence of DIC rather than a cause of DIC due to a potential problem with retrospective study. Prospective cohort studies are needed to determine whether MP-TF activity causes DIC in dogs. Furthermore, MP-TF activity and D-dimer concentration decreased by the time of recovery in some dogs with DIC, suggesting that decreased MP-TF activity reflects the withdrawal from the hypercoagulable state in dogs. Prospective studies with comprehensive monitoring for DIC development over a defined follow-up period are needed to determine whether MP-TF activity is a predictive DIC biomarker.

MP-TF activity was not increased in 4 of 12 dogs (33.3%) with DIC. This could be caused if our assay has a low sensitivity to MP-TF activity. It could also mean that hypercoagulability occurs in the absence of MP-TF activity, leading alternative cellular and molecular pathways to primarily contribute to hypercoagulability. In this regard, several factors like anionic phospholipids, polyphosphates, and nucleic acids have been indicated as key intrinsic contact pathway activation mediators [13]. On the other hand, MP-TF activity was increased in 2 of 14 dogs (14.3%) in non-DIC group. Several reasons could be considered, which were as follows: First, increased MP-TF activity might reflect pre-DIC state before onset of DIC. Second, natural anticoagulant pathways, such as tissue factor pathway inhibitor, antithrombin, protein C, protein S, and thrombomodulin, might prevent onset of DIC in the presence of increased MP-TF activity [15]. Third, several pre-analytical and analytical variables might have affected MP-TF activity [11].

There are several additional limitations to our study. First, there was a very small number of dogs in each group. Second, several factors like sample size, age, gender, dog breed, and disease types were not completely matched among the groups. Third, DIC diagnosis could be imprecise because there is no validated gold standard for DIC diagnosis in dogs. A recent study indicated that thrombin-antithrombin complex (TAT) is a useful marker for DIC diagnosis in dogs [17]. In future studies, TAT concentration should be included for DIC diagnosis. Fourth, concurrent diseases, medications, and other treatments were not considered in this study. Finally, the handling and delay from blood collection to centrifugation varied among samples due to the retrospective nature of this study. This might affect the formation of MPs in samples [11].

In conclusion, this study is the first to suggest that increased MP-TF activity reflects hypercoagulability and is associated with DIC development in dogs with various diseases. However, a conclusion cannot be made regarding the causal relationship between MP-TF activity and DIC development. Larger prospective studies are warranted to determine whether MP-TF activity causes DIC in dogs and to assess its value as a diagnostic and predictive DIC biomarker.

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