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Evaluation of Hemostatic Function with Thromboelastography in Dogs with Hypercoagulable Diseases

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Abstract : Thromboembolic complications are increasing in veterinary medicine. Thromboelastography (TEG) is a more comprehensive method for assessing the clotting process than standard plasma-based coagulation tests. This study compared the ability of TEG and standard coagulation tests to analyze the overall hemostatic state of dogs. The study involved 40 dogs with underlying diseases that predispose to hypercoagulability, including neoplasia, hyperadrenocorticism, immune-mediated diseases, gastrointestinal diseases, and protein-losing nephropathies and enteropathies, and 20 healthy dogs. Their overall hemostatic functional state was evaluated by TEG and routine coagulation assays, including activated partial thromboplastin time, prothrombin time, platelet count, and D-dimer concentration. TEG analysis showed significant differences in clot formation time, α angle, and maximum amplitude (MA) between diseased and control dogs ($P < 0.001$ each). Increased MA was the most frequent abnormality on TEG and was indicative of hypercoagulability. TEG was useful in detecting hemostatic dysfunction in dogs with diseases associated with hypercoagulability. Dogs with TEG tracings indicative of hypercoagulability are likely to be in procoagulant states. Future prospective studies are needed to evaluate whether TEG tracings indicative of hypercoagulability are predictive of thrombosis in dogs.

Key words **:** canine, coagulation, hemostasis, hypercoagulability, thromboelastography.

Introduction

Hypercoagulability is defined as the shifting of the hemostatic balance towards a procoagulant state, including excessive platelet activation and fibrin deposition leading to thrombosis. A hypercoagulable state has been reported in dogs with immune-mediated hemolytic anemia (IMHA), neoplasia, hyperadrenocorticism, protein-losing nephropathies (PLN) and enteropathies (PLE), early disseminated intravascular coagulation (DIC), and sepsis (3,5,8,12,13,14).

Thromboembolic complications are of growing concern in veterinary medicine. Plasma-based coagulation assays have traditionally been used to detect alterations in coagulation status. These assays include determinations of prothrombin time (PT), activated partial thromboplastin (APTT), antithrombin (AT) activity, fibrin degradation products (FDPs), D-dimer, and fibrinogen. Accurate and early detection of a hypercoagulable state using conventional coagulation assays, however, is challenging, in that these assays do not evaluate overall hemostatic capacity and assess only small portions of coagulation pathways (8,13,14). Moreover, conventional coagulation tests, including those for FDPs and D-dimer, provide only non-specific information about hypercoagulability associated with thromboembolic diseases.

In contrast to these traditional plasma-based coagulation tests, thromboelastography (TEG) assesses both the cellular and plasma components of the hemostatic process, providing a comprehensive outline of the clotting process, from initial thrombin generation to formation of fibrin to fibrinolysis. This method allows hypercoagulable states to be identified and quantified (4).

The objective of this study was to compare the results of TEG and standard coagulation tests in dogs with underlying diseases known to predispose to hypercoagulability and in healthy control dogs. In addition, this study was designed to characterize the overall hemostatic state in dogs with hypercoagulable diseases, including neoplasia, hyperadrenocorticism, immune-mediated disease, gastrointestinal disease, PLN, and PLE.

Materials and Methods

Animals

This study analyzed 40 dogs with underlying diseases known to predispose to hypercoagulability, including neoplasia, hyperadrenocorticism, immune-mediated disease, gastrointestinal disease, PLN, and PLE. The control group included 20 dogs, determined to be healthy based on the results of a physical examination, complete blood cell count (CBC), serum biochemistry, and conventional coagulation tests including PT, APTT, and D-dimer. Dogs were excluded if, at the time of presentation, they had received any drug that could have altered TEG results or if they were clinically suspected of having any other concurrent diseases.

IMHA was diagnosed as the presence at first presentation

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of anemia (PCV < 30%) and at least one of the following: persistent autoagglutination, positive Coombs tests, or detection of spherocytes in blood smears (11). Neoplasia, which included several tumor types, was confirmed histopathologically. Hyperadrenocorticism was defined as positivity on both low-dose dexamethasone suppression tests (LDDSTs, 8-h cortisol concentration ≥ 1.5 μg/dL) and ACTH stimulation tests (post-ACTH cortisol concentration > 22 μg/dL). Pancreatitis was diagnosed by the presence of gastrointestinal signs and positivity for canine pancreatic lipase immunoreactivity. Dogs with PLN were eligible for enrollment, if they had a UPC > 2.0 with no evidence of pre- or postrenal proteinuria (3,9). PLE was diagnosed in dogs with (1) a history of gastrointestinal disease (including weight loss, vomiting and diarrhea); (2) panhypoproteinemia (serum albumin < 2.8 g/dL and serum globulin < 2.1 g/dL, with reference ranges 2.8-3.9 and 2.1-4.1 g/dL, respectively); (3) exclusion of hepatic dysfunction; and (4) absence of proteinuria (5).

Sampling

Blood was obtained from all dogs by jugular venipuncture with a 20-G needle and collected directly into sample tubes containing 1 mL EDTA for CBC analysis and 0.5 mL 3.2% sodium citrate (final blood to citrate ratio, 9:1) for coagulation analyses (PT, APTT, and D-dimer). Serum biochemistry and CBC analyses were performed within 5 min of sample collection.

TEG analysis

TEG analysis of whole blood was performed using a TEG 5000 analyzer (Thromboelastograph, Haemonetics Corporation, Braintree, MA, USA), as recommended by the manufacturer. Briefly, TEG Hemostasis System Pins and Cups (Haemonetics Corporation) were placed in the TEG analyzer in accordance with standard operating procedures. Each standard TEG cup was placed in a 37°C prewarmed holder, and 360 μl of whole blood was pipetted, as described previously (1).

Subsequently, the pin was gently lowered into the cup and measurements were initiated. Each TEG analysis was run for 30 min, with continuously obtained data transferred electronically to a personal computer.

Four TEG parameters were evaluated. The reaction time (R), defined as the period of latency from blood withdrawal until the clot begins to form, represents intrinsic clotting and is sensitive to thromboplastin procoagulants. The clotting time (K) is defined as the time to clot formation, measured from the end of R until the clot diameter reached 20 mm. The angle (α) represents the rapidity of fibrin build-up and cross-linking, and is mainly dependent on the concentrations of platelets, fibrinogen, and clotting factors. The maximum amplitude (MA) is a direct function of fibrin and platelet binding, and represents the ultimate strength of the fibrin clot. About 80% of MA is dependent on platelet number and function (6,10). The clinical applicability of the TEG analysis was assessed by comparing the ranges of R, K, α, and MA in clinically healthy dogs and dogs with hemostatic abnormalities.

Coagulation tests

Routine coagulation assays included those for platelet counts, PT, APTT, and D-dimer concentration. PT and APTT were evaluated using an automated coagulation analyzer (VetScan VSpro, Abaxis, US). Platelet counts were determined with an automated CBC instrument (MEK-6450K, Nihon Kohden, Rosbach v.d.H., Germany) and D-dimer concentration by an immunometric assay using the gold-antibody conjugate principle.

Statistical analysis

The results of TEG and standard coagulation tests in dogs with a hypercoagulable disease and control dogs were each compared using Welch's t-tests. For all tests, a *P*-value < 0.05 was considered statistically significant. Correlations between TEG parameters and other coagulation and hemostatic variables were assessed by Pearson correlation analysis. Statistical software® was used for all statistical analyses.

Results

The 40 dogs with hypercoagulable diseases included 12

Table 1. Disease conditions in dogs with concurrent diseases known to predispose to hypercoagulability

TEG Results	Disease Category	Specific diseases
Hypercoagulable disease (n = 40)	Neoplasia (18)	Lymphoma (3)
		Mast cell tumor (2)
		Squamous cell carcinoma (1)
		Nasal adenocarcinoma (1)
		Nasal sarcoma (1)
		Mammary gland adenocarcinoma (1)
		Transitional cell carcinoma (1)
		Hepatocellular carcinoma (3)
		Sertoli cell tumor (1)
	Hyperadrenocorticism (9)	Pituitary dependent hyperadrenocorticism
	Gastrointestinal disease (3)	Pancreatitis
	Immune-mediated disease (7)	Immune-mediated hemolytic anemia (4)
		Immune-mediated polyarthritis (3)
	PLN (1)	
	PLE (2)	

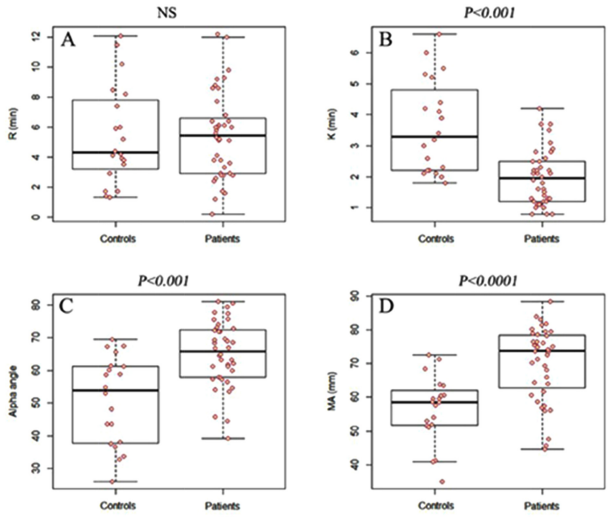

Fig 1. Dot plots of thromboelastography results in dogs with a hypercoagulable disease (n = 40) and in control dogs (n = 20). (A) Reaction time (R); (B) Clot formation time (K); (C) α angle; and (D) Maximum amplitude (MA).

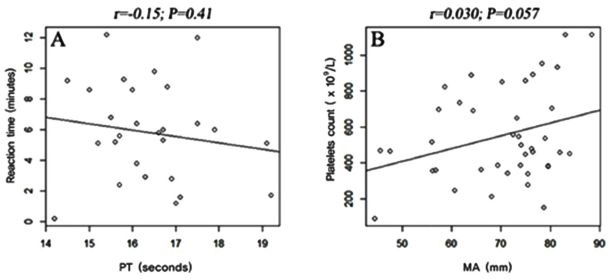

Fig 2. Correlations between reaction time (R) and prothrombin time (PT) ($r = -0.15$, $P = 0.41$) (A), and between platelet count and maximum amplitude (MA) ($r = 0.030$, $P = 0.057$) (B) in dogs with hypercoagulable diseases.

males (7 castrated, 5 intact) and 28 females (12 spayed, 16 intact), ranging in age from 1 to 16.8 years. Breeds represented included Yorkshire Terrier (n = 8), Shih Tzu (n = 7), Maltese (n = 7), Cocker Spaniel (n = 4), Toy Poodle (n = 4), mixed breed (n = 4), Schnauzer (n = 2), Bulldog (n = 1), Pekingese (n = 1), Pomeranian (n = 1), and White Terrier (n = 1). The control dogs included Maltese (n = 6), Pomeranian (n = 3), Shih Tzu (n = 2), Great Pyrenees (n = 2), mixed breed (n = 2), Alaskan Malamute (n = 1), Golden Retriever (n = 1), Beagle (n = 1), Yorkshire Terrier (n = 1), and Chihuahua (n = 1). Of the 40 dogs with hypercoagulable diseases, 18 had various neoplasia, 9 had hyperadrenocorticism, 7 had immune-mediated diseases, 3 had gastrointestinal diseases, 2 had PLE, and 1 had PLN (Table 1). Of the 40 dogs with diseases, 29 also underwent coagulation tests (PT, APTT, and D-dimer).

Significant between group differences were observed in both TEG and traditional coagulation parameters. On TEG traces, K was significantly lower ($P < 0.001$) and α ($P < 0.001$) and MA ($P < 0.001$) significantly higher in the 40 dogs with hypercoagulable diseases than in the 20 healthy control dogs (Fig 1). R, however, was similar in the two groups ($P = 0.9412$). On traditional coagulation tests, platelet count ($P < 0.001$) and D-dimer concentration ($P = 0.032$) were significantly higher and PT significantly lower ($P < 0.001$) in dogs with hypercoagulable diseases than in healthy dogs (Table 2).

No significant positive correlation was observed between R and PT ($r = -0.15$, $P = 0.41$) or between platelet count and MA ($r = 0.030$, $P = 0.057$) (Fig 2).

Discussion

Coagulation abnormalities are frequently encountered in dogs with concurrent diseases associated with hypercoagulability. Lack of early treatment may increase the risks of thromboembolic complications, which are more difficult to manage than coagulation abnormalities and are closely associated with high mortality rates (3,5,8,12,13,14).

Identifying a hypercoagulable state by traditional laboratory coagulation tests is difficult, time-consuming, and costly because the causes of hypercoagulability are multifactorial. Thus, considerable experience and expertise are needed to determine the comprehensive coagulation status in a dog as well as to predict the risks of thromboembolic events (13).

As TEG can detect hypercoagulability, it can be used to evaluate the overall hemostatic state of dogs. This is especially useful in dogs suspected of being at risk of thromboembolic complications due to pathophysiologic conditions that predispose to a hypercoagulable state.

Our study is the first to show that TEG can detect hypercoagulability in a group of dogs with hypercoagulable diseases. TEG parameters indicative of hypercoagulability include significantly shorter K, and significantly increased α and MA. These findings indicate that hypercoagulable diseases result in both increased clotting activity and greater final clot strength.

Several conclusions can be drawn from our results. First, several of these dogs with a hypercoagulable state simultaneously had prolonged APTT that was closely associated

Table 2. Results of standard coagulation tests in dogs with a hypercoagulable disease and in control dogs

Standard coagulation test (Reference)	Diseased dogs (n = 29)	Control dogs (n = 20)	P-value
Platelet (120-600 × 10^6/μL)	552.2 (90-1116)	384.3 (255-560)	< .001
PT (14-19 s)	16.44 (14.2-19.2)	18.1 (16.8-22.9)	< .001
APTT (75-105 s)	103.58 (79-130.8)	106.4 (87.5-135.1)	0.416
D-dimer (< 0.3 μg/mL)	0.32 (0.1-1.8)	0.15 (0.1-0.4)	0.032

with hypocoagulability. These contradictory results suggest that plasma-based coagulation assays can only assess single components of comprehensive coagulation pathways, making them incorrect determinants of hypocoagulability. In contrast, TEG measurements are designed specifically to assess comprehensive clotting kinetics and strength in whole blood. TEG may therefore play a valuable role in assessing and predicting prothrombotic tendencies in patients with hypercoagulable diseases.

Second, previous studies in humans have reported that increased MA, reflecting final clot strength, is highly associated with platelet activation and fibrin binding (1,5,12). Postoperative TEG analysis indicated that high MA correlated with a high risk of hypercoagulability. Hypercoagulability plays a crucial role in the pathogenesis of thromboembolic complications, including myocardial infarction, ischemic stroke, deep vein thrombosis, and pulmonary embolism. Interestingly, our study found that several dogs with increased MA, indicative of a hypercoagulable state, had platelet counts within the reference range. Some dogs with underlying diseases associated with hypercoagulability showed no correlation between MA and platelet counts, a finding inconsistent with previous results (1,5,12). These results indicate that dogs with hypercoagulable diseases had complicated hemostatic disorders and that TEG may be particularly useful in assessing their global hemostatic capacities.

We found that R value was similar in dogs with hypercoagulable diseases and in control dogs ($P = 0.94$). This finding suggest that coagulation pathways including the intrinsic pathway and initial fibrin formation may not contribute to hypercoagulability as much as other coagulation parameters (9).

Previous studies evaluating the association between platelet hyperactivity and hypercoagulability found that dogs with malignant neoplasia showed a hemostatic tendency toward platelet hyperaggregability. This was similar to results observed in human cancer patients, who showed evidence of hypercoagulability and were at increased risk of developing complications including deep venous thrombosis (2).

Although dogs with hypercoagulable diseases are likely more prone to develop thromboembolic events, additional studies are needed to assess whether these dogs develop thromboembolic diseases warranting treatment with anticoagulants. Many attempts have been made to use TEG to predict thromboembolic events (2,8). Among the TEG parameters, MA seemed to be the most suitable for detecting a hypercoagulable state. However, no consensus has been reached on a clinically applicable gold standard to predict thromboembolic events in high-risk patients (2,7).

Further prospective studies are needed, including studies using larger numbers of dogs with hypercoagulable diseases. In addition, efforts should be made to correlate the results of TEG tracings with clinical signs of procoagulation and hypercoagulable states implicated in the pathogenesis of thromboembolism. Furthermore, TEG may be valuable in characterizing coagulation abnormalities in patients at risk of thrombosis. This may enhance early and accurate clinical decisions facilitating individually tailored preventive management, early adoption of anticoagulant treatment protocols, resulting in the minimization of thrombotic complications in veterinary medicine.

In summary, TEG analysis showed that a majority of dogs with hypercoagulable diseases have coagulation abnormalities, with the most common hemostatic dysfunction being hypercoagulability, similar to results in humans. These findings indicate that dogs with hypercoagulable diseases and hypercoagulability may serve as a clinical model for the human disease. This newly validated TEG method may provide important information for optimizing therapeutic interventions with the minimum risk of thromboembolic complications. Additional studies are warranted to design a TEG based multifocal approach to assess comprehensive hemostatic balance and thrombosis in dogs as well as in humans.

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References

1. Bauer, Natali B, Eralp O, Moritz A. Effect of hemolysis on canine kaolin-activated thromboelastography values and ADVIA 2120 platelet activation indices. Vet Clin Pathol 2010; 39: 180-189.
2. Dai Y, Lee A, Critchley LA, White PF. Does thromboelastography predict postoperative thromboembolic events? A systematic review of the literature. Anesth Analg 2009; 108: 734-742.
3. Donahue, Suzanne M, Brooks M, Otto CM. Examination of hemostatic parameters to detect hypercoagulability in dogs with severe protein-losing nephropathy. J Vet Emerg Crit Care 2011; 21: 346-355.
4. Donahue Suzanne M, Otto CM. Thromboelastography: a tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. J Vet Emerg Crit Care 2005; 15: 9-16.
5. Goodwin L, Goggs R, Chan D, Allenspach K. Hypercoagulability in dogs with protein-losing enteropathy. J Vet Intern Med 2011; 25: 273-277.
6. Koenigshof AM, Scott MA, Brown AJ. Effects of delayed anticoagulation and use of evacuated tubes on non-activated thrombelastography in dogs. Vet Clin Pathol 2012; 41: 63-70.
7. Kol A, Nelson R, Gosselin R, Borjesson D. Characterization of thrombelastography over time in dogs with hyperadrenocorticism. Vet J 2013; 197: 675-681.
8. Kristensen AT, Wiinberg B, Jessen LR, Andreasen E, Jensen AL. Evaluation of human recombinant tissue factor-activated thromboelastography in 49 dogs with neoplasia. J Vet Intern Med 2008; 22: 140-147.
9. Lennon E, Hanel R, Walker J, Vaden S. Hypercoagulability in dogs with protein-losing nephropathy as assessed by thromboelastography. J Vet Intern Med 2013; 27: 462-468.
10. Mallett SV, Cox DJ. Thromboelastography. Brit J Anaesth 1992; 69: 307-313.
11. Sinnott VB, Otto CM. Use of thromboelastography in dogs with immune-mediated hemolytic anemia: 39 cases (2000-2008). J Vet Emerg Crit Care 2009; 19: 484-488.
12. Trapani LM. Thromboelastography: current applications, future directions. Open J Anesthesiol 2013; 3: 23-27

13. Wagg CR, Boysen SR, Bédard C. Thrombelastography in dogs admitted to an intensive care unit. Vet Clin Pathol 2009; 38: 453-461.
14. Wiinberg B, Jensen AL, Johansson P, Rozanski E, Tranholm M, Kristensen AT. Thromboelastographic evaluation of hemostatic function in dogs with disseminated intravascular coagulation. J Vet Intern Med 2008; 22: 357-365.