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Whole-blood thrombelastography using calcium chloride activation in healthy cats

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Abstract: Thrombelastography (TEG, Haemoscope Corp., Niles, IL, USA) allows for a global evaluation of the hemostatic system; while conventional coagulation tests typically evaluate only one part of the coagulation system, TEG simultaneously examines the interaction between platelets, clotting factors, the fibrinolytic system, and clot retraction mechanisms. Since its development, TEG has been widely employed in human clinical medicine and research, but has only recently gained popularity in veterinary medicine. The purpose of this study was to establish TEG reference ranges in healthy cats using the citrated native technique. In this study, 31 clinically healthy cats were evaluated. We sampled healthy adult cats based on the absence of clinical signs of illness, normal physical examination findings, a complete blood count, hemostasis profile results, and no previous history of bleeding disorders. The cats had 2 distinct tracings: a "normal" tracing similar to that obtained in other species, and a "high lysis" tracing. The percent of lysis at 60 min (LY60) was significantly higher and the percent of lysis at 60 min after MA is reached (CL60) was significantly lower in the "high lysis" group ($P < 0.001$ for both). Cats in the "high lysis" group also had a significantly shorter reaction time ($P = 0.02$). Based on the results, citrated native TEG may provide valuable information on global hemostasis in cats. This technique has a high coefficient of variation for the reaction time, kinetic time, and LY60 parameters, likely due to platelet retraction. It should be useful for detecting hypo- and hypercoagulable states in cats with hemostatic disturbances.

Key words: Thrombelastography, hemostasis, viscoelasticity, cats

1. Introduction

Hemostasis is a complex and dynamic process. Several clinical entities such as hypertrophic cardiomyopathy (HCM), vitamin K deficiency, liver disease, neoplasia, feline infectious peritonitis, disseminated intravascular coagulation, sepsis, and surgical complications can be associated with clinically relevant hemostatic abnormalities (1,2).

Several standard tests are used to evaluate and monitor patients with hemostatic disorders, including platelet count and platelet function assays using a platelet function analyzer (PFA-100, Siemens, Germany), one-stage prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen concentration (FIB), among others (3–5). In veterinary medicine, thrombelastography (TEG) can be used to assess the hemostatic system in several clinical and laboratory settings (6). TEG has also been used to guide the transfusion of blood components in human patients undergoing cardiac surgery or liver transplantation (7,8). This assay has been used for a variety of clinical purposes, and with the recent introduction of improved equipment and software the spectrum of clinical applications of TEG analysis has increased markedly (9). However, to our knowledge, only a few studies of TEG in cats have been published thus far (10–12), and the value of TEG testing in cats with hemostatic disorders is still unclear.

In cats, thromboembolism associated with HCM is a relatively common emergency (13–15). Affected cats develop arterial thromboembolism as a consequence of a systemic hypercoagulable state or endothelial injury (14). In cats with HCM, there is an increased risk of thrombus formation due to low flow in an enlarged left atrium, leading to aortic or other systemic thromboembolic complications (14,16,17). An enlarged left atrium could also contribute to intracardiac thrombus formation by impaired clearance of

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activated coagulation factors and enhancement of platelet-endothelial interactions (18). Hypercoagulability is also likely to occur in cats with neoplastic and inflammatory disorders. Hemostatic disorders reported in cats include disseminated intravascular coagulation, hemophilia, hepatic failure, and vitamin K deficiency associated with viral, bacterial, protozoal, and parasitic infections, as well as trauma, burns, and liver disease (1,2,14,19–21).

The tests of hemostasis commonly used in veterinary medicine, including APTT, PT, TT, FIB, factor VIII, thrombin-antithrombin complex, D-dimer, fibrin degradation products (FDPs), antithrombin, protein C, plasminogen, tissue plasminogen activator, alpha-2 antiplasmin, and plasminogen activator inhibitor, evaluate different aspects of the coagulation and fibrinolytic system, but assessment of hypercoagulability is not easily accomplished using these conventional laboratory methods (3–5). To our knowledge, these traditional assays cannot identify hypercoagulability before the development of thrombosis. However, it appears that TEG can detect this prethrombotic state. In humans, TEG is more sensitive than routine coagulation assays for the detection of a hypercoagulable state (6,22).

TEG is a useful tool for recording viscoelastic changes in whole blood during coagulation (23,24), including clot formation, kinetics, strength, stability, and resolution (3). For this reason, TEG is likely the ideal instrument to evaluate hyper- and hypocoagulable states, fibrinolysis, clot strength, and anticoagulant drug effects in several clinical and laboratory settings.

TEG allows for a global evaluation of the hemostatic system; while conventional coagulation tests typically evaluate only one part of the coagulation system, TEG also assesses the function of both cellular and soluble components of the hemostatic system. TEG also simultaneously examines the interaction among platelets, clotting factors, the fibrinolytic system, and clot retraction mechanisms (3,24,25). Since its development, TEG has been widely employed in human clinical medicine and research, but has only recently gained popularity in veterinary medicine (6,26,27).

The purpose of this study was to establish TEG reference ranges in clinically healthy cats using the whole-blood citrated native technique.

2. Materials and methods

Thirty-one clinically healthy cats were evaluated. These cats were either enrolled as blood donors in the Ohio State University Animal Blood Bank (OSUABB) or into the study after signed owner consent. The OSUABB has a current animal use protocol on file. The use of client-owned cats with signed owner consent was approved by the Veterinary Teaching Hospital Board.

Cats were considered healthy based on the absence of clinical signs of illness, normal physical examination findings, a complete blood count (CBC), hemostasis profile (PT, APTT, and fibrinogen) results, and no previous history of bleeding disorders.

The sample population ranged in age from 1 to 7 years old (mean = 3.6 years). Breeds included Domestic Shorthair (n = 25), Domestic Longhair (n = 3), American Shorthair (n = 1), Persian (n = 1), and Himalayan (n = 1). Weights of the sampled cats ranged from 3.8 kg to 6.8 kg with a mean weight of 5.5 kg.

2.1. Sample collection

Samples were obtained from non-sedated cats via medial saphenous venipuncture using a 21-gauge butterfly catheter (SURFLO® winged infusion set, Terumo Corp.). All blood samples were obtained from the butterfly catheters in tandem using 3-mL sterile syringes (MonoJect, Tyco Healthcare) to provide gentle negative pressure during collection. Using a sterile syringe, 0.5–1.0 mL of blood was collected and immediately transferred into a collection tube containing EDTA anticoagulant for blood smear preparation and CBC using a LaserCyte (IDEXX Laboratories, Westbrook, ME, USA).

For all hemostasis assays, a 3-mL sterile syringe was prepared with 0.3 mL of 3.2% buffered sodium citrate anticoagulant prior to blood collection. In order to reach a final 1:9 proportion of anticoagulant to blood, 2.7 mL of blood was collected in the prepared syringes and gently mixed before being transferred into a collection vial. The remaining blood was centrifuged at 1380 × *g* for 10 min to obtain plasma, and this was stored at –30 °C for the hemostasis panel. Finally, 2 microhematocrit tubes were filled by capillarity action from the residual native blood remaining in the butterfly catheter immediately following venipuncture and spun to obtain a packed cell volume (PCV).

2.2. TEG analysis

Citrated whole blood samples were stabilized for 30–40 min at room temperature prior to being recalcified and analyzed in the TEG-5000 (Haemoscope Corp., Niles, IL, USA) according to the manufacturer's instructions. A single TEG test per sample was performed as follows: 340 µL of citrated whole blood (CWB) was mixed with 20 µL of $CaCl_2$ within the reagent cup of the TEG and traces were obtained after 120–180 min of running time at 37 °C.

2.3. Statistical analysis

All data were evaluated for normality using the D'Agostino test and descriptive statistics were taken. TEG parameters, PCV, and age were compared between the "normal" and "high-lysis" groups using non-parametric methods (Mann–Whitney). The Prism statistical software package was used to analyze the data.

3. Results

The results of TEG in the 31 healthy cats, expressed as means ± standard deviations are presented in Table 1. By visually inspecting the TEG tracings, we identified 2 distinct tracings: a "normal" tracing (n = 24) similar to that obtained in other species, and a "high lysis" tracing (n = 7) (Figure 1). The percent of lysis at 60 min (LY60) was significantly higher and the percent of lysis at 60 min after MA is reached (CL60) was significantly lower in the "high lysis" group ($P < 0.001$ for both). Cats in the "high lysis" group also had significantly shorter reaction times ($P = 0.02$) (Table 2, Figure 2).

4. Discussion

Hemostasis is regulated by a delicate balance between pro- and anti-coagulant factors and the effects of fibrinolysis. Thrombelastography is a clinically applicable global hemostasis assay that provides information about each component of this process, both singly and in combination (9,28). Whole blood coagulation assays are a novel method of studying hemostasis in an environment that allows for interaction between cellular and enzymatic components of coagulation (23,29). To date, TEG has been used to diagnose and manage animals and humans with hemostatic disorders in various clinical situations (9,30).

Abnormalities of hemostasis are common contributors to morbidity and mortality in small animals. Unfortunately, conventional tests of hemostasis do not fully identify individual clotting defects (31,32). Routine coagulation tests are plasma-based, whereas the TEG assay is an assay based on whole blood that includes both cellular and plasma components important for initiation, amplification, propagation, and lysis of the forming blood clot (6). An important advantage of TEG over conventional hemostasis testing is that it is a dynamic method that yields information relating to the cumulative and global effects of the many components of coagulation, including platelet function (33).

The parameters routinely evaluated on a TEG tracing include reaction time (R), kinetic time (K), angle (α), maximum amplitude (MA), time to maximum amplitude (TMA), viscoelasticity (G), and percent lysis at 60 min (LY60). CL60 shows fibrinolytic status at 60 min after MA is reached. R is calculated from the time of addition of the agonist to the sample until the clot formation begins; this value represents the enzymatic portion of coagulation, which is brought about by the action of clotting factors. K is calculated from the end of R until the clot reaches a predetermined firmness; this value therefore is a measure of the speed at which a certain level of clot strength is reached, representing clot kinetics. The α value provides an assessment of the strengthening of the clot through fibrin formation and cross-linking; therefore, this value is also related to the kinetics of clot formation. The maximum amplitude (MA) is a measure of the ultimate strength of the fibrin clot and primarily represents a measure of platelet function. The G value provides a measure of clot strength and viscoelasticity in dyne/cm^2 and is calculated from the MA. Finally, LY60 represents the proportion of clot lysis, measuring both clot retraction and fibrinolysis, by comparing the decrease in amplitude from the MA to the amplitude measured at 60 min (27).

Based on this study, the TEG results had acceptable precision. The coefficients of variation (CVs) for intra-assay variability in healthy cats were: R = 59.8%, K = 66.9%, α = 25.6%, MA = 17.2%, TMA = 26.3%, G = 35.5%, CL60 = 18.4%, and LY60 = 109% (Table 1). There were low intra-assay CVs for α, MA, TMA, and G; however, R, K, CL60, and LY60 had significant intra-assay variability. This variability may be due to the low number of samples and high interindividual variability or to breed variability, daily variation, or storage time (25,34). The CL60 and LY60 results are likely associated with the 2 distinct "fibrinolytic" patterns we recognized (as clarified in the next paragraph). The variations in the TEG parameters are probably not due to operator handling, as all TEG assays were done by the same individual using standardized procedures.

In our study, the MA values had the lowest CV (17.2%) and intra-assay variation was also minimal, which suggests that it could be the most consistent and reliable parameter for establishing reference ranges in cats. Conversely, we found a high CV for LY60 (109%). As discussed in the results, we identified 2 types of TEG tracings in normal cats: a "normal" tracing similar to those in other mammalian species, and a "high lysis" group characterized by an abrupt increase in lysis within 30–60 min (Figure 1). When evaluated as separate subgroups, the mean LY60 in the "normal" population was 4.8 ± 3.6%, with a CV of 83%, whereas the mean LY60 in the "high lysis" group was 27 ± 8.6%, with a CV of 32.7% ($P < 0.001$). Interestingly, R was significantly shorter in the cats with high lysis tracings (Table 2).

Percent lysis at 30 min (LY30) and LY60 values are typically attributed to enhanced fibrinolysis; however, Katori et al. (35) recently reported the occurrence of clot retraction as a result of a "hyperactive platelet function" in humans. Given the fact that cat platelets are frequently considered to be hyperreactive (17,36), this is a more plausible theory for the increased LY60 in this subset of samples. Preincubation of the sample with abciximab, a platelet aggregation inhibitor that works by blocking the fibrinogen receptor glycoprotein IIa/IIIb, should confirm whether this pattern is due to platelet retraction or hyperfibrinolysis (35). Interestingly, the shorter R in the cats with "high lysis" tracing is compatible with hypercoagulability, whereas high fibrinolysis is associated

Table 1. TEG values and hemostasis profiles for 31 healthy cats.

	R (min)	K (min)	α (°)	MA (mm)	TMA (min)	G (d/s)	CL60 (%)	LY60 (%)	PT (s)	APTT (s)	FIB (mg/dL)	PLT ($\times 10^3$)	Age (years)	Weight (kg)	PCV (%)
Minimum	1.1	1.2	19.9	30.4	13.4	2180	44.3	0.1	8.3	8.3	59.0	95	1	4.1	32
Median	4.2	2.4	55.3	52.2	23.2	5451	83	7.1	9.5	11.3	106	245	4	5.7	42
75th percentile	6.1	3.4	64.3	61	29.4	7826	92.6	12.1	10.7	13	129	344	5	6.3	44
Maximum	13.2	10.2	72.7	66.3	39.4	9853	98.3	37.4	12.6	14.9	165.0	568	7	6.8	50
Mean	4.7	3.1	53.1	53.7	24.4	5947	80.1	9.8	9.8	11.4	107.4	253	3.9	5,7	41
Std. Deviation	2.9	2.1	13.6	9.1	6.4	2110	14.9	10.7	1.0	1.9	27.9	112.2	1.7	0.7	4.9
Std. Error	0.5	0.4	2.4	1.6	1.2	379	2.7	1.9	0.2	0.3	5.0	20.2	0.4	0.1	1
Lower 95% CI of mean	3.7	2.3	48.1	49.3	22.1	5173	75.4	5.9	9.4	10.7	97.2	211.9	3.2	5.4	38.9
Upper 95% CI of mean	5.8	3.8	58.1	56	26.8	6721	86.3	13.7	10.2	12.1	117.7	294.2	4.6	6	43.2
Coefficient of variation (%)	59.81	66.85	25.63	17.23	26.32	35.48	18.38	109	10.2	16.7	25.9	44.3	43.5	12.5	11.9

R = reaction time; K = kinetic time; α = angle; MA = maximum amplitude; CL60 = lysis (fibrinolytic status) 60 min after MA is reached; LY60 = clot lysis 60 min after MA is reached; TMA = time to maximum amplitude; G = viscoelasticity; PT = prothrombin time; APTT = activated partial thromboplastin time; PLT = platelet; FIB = fibrinogen; PCV = packed cell volume; and TEG = thrombelastography.

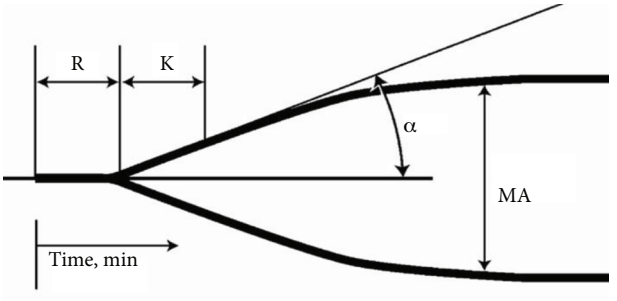

Figure 1. A typical thromboelastogram with the following measurements represented: R, K, α, and MA.

with hypocoagulability. This also supports platelet hyperreactivity as the cause of the high LY60 (Table 2).

Hemostasis and coagulation tests, like all other laboratory tests, are subject to both analytical variations, which are dependent on the laboratory methods used, and biological variations within and between individuals (37). In veterinary medicine, there are studies evaluating TEG tracings in rats (38,39), rabbits (40), guinea pigs (41), cows (42), sheep (43), cats (10–12), dogs (44), and fish (45). Reference ranges have been established for adult dogs using different activators (6,27,37).

TEG has been performed in a study investigating anticoagulant effects of low-molecular-weight heparin in cats (10); as in our study, their TEG was done with native recalcified citrated whole blood. Different activators such as kaolin, tissue factor/kaolin, and celite provide the opportunity to investigate the role of contact proteins or tissue factors in initiating the clotting process. However, such approaches lead to difficulty in establishing standards and reference values (46).

In a previous study, Alwood et al. (10) proposed reference ranges for 25 healthy cats to be 1.5–4.4 min for R, 1.0–2.8 min for K, 59.2–79.8° for α, and 46.0–69.2 mm for MA. These reference values are similar to those found in our study.

Marschner et al. (11) performed 90 TEG analyses on citrated whole blood samples from 15 clinically healthy cats using assays without an activator (native) or with human recombinant tissue factor (TF) or kaolin as activators. Their CVs were highest in the native assay and comparable in the TF and kaolin activated assays. Significant differences were determined between native and kaolin assays for all measured parameters, between kaolin and TF for all measured parameters except LY60, and between native and TF assays for R and K.

In this study, we evaluated TEG tracings and parameters in 31 normal cats and the general pattern was similar to those of other species, with some differences. The main difference between TEG tracings in cats and other mammalian species is the high LY60; however, when the tracings are divided in 2 subgroups, the LY60 in "normal" cats is similar to that in dogs and horses. Using $CaCl_2$ activation, the mean LY60 values in dogs were 3.1 ± 2.5% in non-greyhounds and 2.8 ± 5% in greyhounds (27), and in horses they were 3.2 ± 2.5% (47). The "normal" cat TEG tracings had a mean LY60 value of 9.8 ± 10.7%.

Disorders of hemostasis are frequently found in cats and if not diagnosed and treated early may lead to various complications and death (21). We lack an adequate means to monitor treatment efficacy using the traditional methodology. The overall hemostatic state most commonly observed in cats with HCM is hypercoagulability (2,15). The continuous monitoring of hypercoagulability during the course of the disease and assessing therapeutic intervention is difficult with current methods (4). In other species, TEG tracings reveal hypercoagulability prior to the appearance of clinical signs (24). The value of TEG as an early and precise predictor of coagulation disturbances, especially hypercoagulability, has been assessed by investigation of the systematic changes caused by different

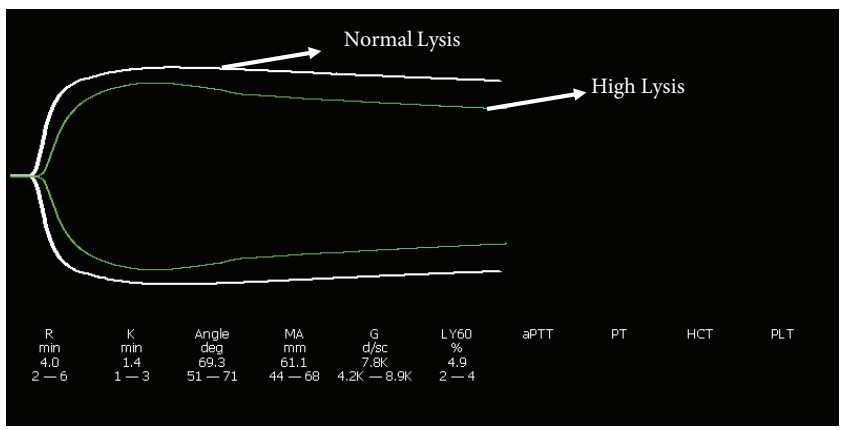

Figure 2. Thromboelastograms from 2 different cats with normal and high lysis.

Table 2. TEG values and hemostasis profiles for the normal tracing group (NTG, n = 24) and the high lysis tracing group (HTG, n = 7).

Groups	Median ± SE		Mean ± SD												
	R (min)	K (min)	α (°)	MA (mm)	TMA (min)	G (d/s)	CL60 (%)	LY60 (%)	PT (s)	APTT (s)	FIB (mg/dL)	PLT ($\times 10^3$)	Age (years)	Weight (kg)	PCV (%)
NTG	4.6 ± 0.6	2.7 ± 0.4	50.8 ± 14.4	51.8 ± 9.7	25.7 ± 6.6	5797 ± 2207	87.7 ± 6.5	4.78 ± 3.6	9.8 ± 0.9	11.4 ± 1.9	108.9 ± 28.4	246.6 ± 98.3	2.2 ± 1.3	5.6 ± 0.5	42.4 ± 5.9
HTG	3.4 ± 0.3	2 ± 0.2	61 ± 6.4	55.6 ± 6.1	20.2 ± 3.2	6460 ± 1785	57.49 ± 10.8	27 ± 8.6	9.7 ± 1.5	11.4 ± 2.0	102.4 ± 27.6	275.1 ± 158.4	4.3 ± 1.5	5.4 ± 1.1	38.6 ± 2.4

R = reaction time; K = kinetic time; α = angle; MA = maximum amplitude; CL60 = lysis (fibrinolytic status) 60 min after MA is reached; LY60 = clot lysis 60 min after MA is reached; TMA = time to maximum amplitude; G = viscoelasticity; PT = prothrombin time; APTT = activated partial thromboplastin time; PLT = platelet; FIB = fibrinogen; PCV = packed cell volume; and TEG = thrombelastography.

coagulation disorders (22,48). Hypercoagulability should be associated with decreased R and K values, increased MA, and increased α (49). For this reason, TEG testing may be a suitable tool to predict hypercoagulable states in cats with HCM with arterial thromboembolism events.

Furthermore, in our hospital, a complete hemostasis screening costs $160–200 while the cost of a TEG assessment of a whole-blood hemostasis is $45 including the cost of consumables, a technician's time, and hardware depreciation.

To our knowledge, this is the first study that describes the characteristics of the normal feline TEG, which differs in part from that of other species. This study will make comparisons of results from diseased cats easier in the future.

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