

Kaolin-activated thromboelastography in echocardiographically normal cats

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Objective—To determine reference values for kaolin-activated thromboelastography in echocardiographically normal cats.

Animals—30 healthy cats without evidence of cardiomyopathy on echocardiographic examination.

Procedures—All cats underwent echocardiographic examination, the findings of which were reviewed by a board-certified cardiologist. Cats that struggled ($n = 10$) received mild sedation with butorphanol and midazolam IM to permit phlebotomy without interruption in jugular venous blood flow. Blood samples were collected for analysis of thromboelastography variables, PCV, total solids concentration, platelet count, activated partial thromboplastin time, prothrombin time, fibrinogen concentration, and antithrombin concentration.

Results—All 4 thromboelastography variables had $< 5\%$ mean intra-assay variability. Mean values were as follows: reaction time, 4.3 minutes; clotting time, 1.6 minutes; α angle, 66.5° ; and maximum amplitude, 56.4 mm. Compared with nonsedated cats, cats that required sedation had a significantly shorter clotting time and greater α angle, whereas reaction time and maximum amplitude were not significantly different.

Conclusions and Clinical Relevance—Kaolin-activated thromboelastography was a reliable test with unremarkable intra-assay variability in echocardiographically normal cats. Sedation may affect certain thromboelastography variables, but the effect is unlikely to be clinically important. It remains unknown whether subclinical cardiomyopathy has a significant effect on thromboelastography variables in cats. (*Am J Vet Res* 2012;73:775–778)

Thromboelastography is an in vitro test of global hemostatic function including clot formation, clot strength, and subsequent fibrinolysis, providing information not available through traditional coagulation testing. The method is not new and has been used for decades in human medicine as a point-of-care test of whole blood during cardiac and liver transplantation.^{1,2} In such circumstances, TEG must be performed within 4 to 6 minutes after blood collection. Thromboelastography can also be performed on recalcified citrated whole blood with an activator such as tissue factor or kaolin added 30 to 90 minutes after blood collection to assist coagulation, making this technique practical for use in veterinary clinical settings.

In dogs, reference intervals for TEG variables have been established,^{3,4} and TEG results have been used to identify important coagulation abnormalities in dogs with parvoviral enteritis, neoplasia, disseminated intravascular coagulation, and immune-mediated hemolytic anemia.^{3–9} Similar studies are lacking in cats, in which reliable TEG reference intervals have not been consistently established. At the authors' and oth-

ABBREVIATIONS	
MA	Maximum amplitude
TEG	Thromboelastography

ers' institutions, demonstration of hypercoagulability when TEG is used in animals with certain critical diseases may be resulting in increasing use of antithrombotic treatments.^a Whether such practice will result in improved outcomes in these situations remains to be seen.^{6,9}

Thromboelastography has been performed in healthy cats in several studies^{10,11,b,c} by use of recalcified citrated whole blood as well as tissue factor- and kaolin-activated whole blood. Cats in those studies were judged healthy through physical examination and routine blood testing but were not screened for occult cardiomyopathy through echocardiography. Investigators in another study¹² used echocardiography to identify cardiomyopathy in 16% of apparently healthy cats. Of those cats with cardiomyopathy, only a third had a heart murmur when examined by a board-certified veterinary cardiologist. Given that cardiac disease can affect coagulation in humans, dogs, and cats,^{13–16} inclusion of cats with subclinical cardiomyopathy in a group chosen for determination of TEG reference intervals could lead to inaccurate results.

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A recent study¹⁰ involving the use of TEG on recalcified citrated whole blood from clinically normal cats revealed considerable intra-assay variability, with 15 of 40 samples excluded from analysis because of > 20% variability for ≥ 2 variables. A study¹⁶ of hypercoagulability in cats with cardiomyopathy showed a similar degree of difficulty in obtaining reliable results from coagulation testing, leading to exclusion of some results.^d It was subsequently postulated that such findings may have been the result of an alteration in platelet activation and initiation of the coagulation cascade attributable to systemic catecholamine release and blood collection techniques.

The primary objective of the study reported here was to determine TEG values for kaolin-activated whole blood from echocardiographically normal cats. A secondary aim was to determine whether a specific venipuncture and blood collection technique, coupled with mild sedation when necessary, would result in minimal intra-assay variability of dual TEG tracings in the same cats.

Materials and Methods

Cats—Forty clinically healthy staff- or student-owned cats were initially included in the study. Two-dimensional and M-mode echocardiographic examination was performed by a board-certified veterinary cardiologist (JR) or by another veterinarian, in which situation the veterinary cardiologist reviewed the findings. Standard echocardiographic M-mode measurements were obtained, including the interventricular septal thickness during diastole and systole, left ventricular internal diameter during diastole and systole, and posterior wall thickness during diastole and systole. The aorta and left atrial diameter were measured on the short-axis view obtained through the right parasternal window as described elsewhere.¹⁷

Cats that struggled during echocardiography and thus were considered likely to struggle during phlebotomy were sedated with butorphanol (0.2 mg/kg, IM) and midazolam (0.2 mg/kg, IM) and allowed to sit quietly for an additional 30 minutes prior to blood collection. Cats that strongly resisted echocardiography or blood collection technique despite sedation were excluded from the study. This study was approved by the Clinical Studies Review Committee at Tufts University Cummings School of Veterinary Medicine.

Blood sample collection—Blood samples were collected via jugular venipuncture with a 21-gauge butterfly needle into evacuated serum separator tubes, plastic tubes containing 3.2% sodium citrate, and tubes containing EDTA, in that order.^{18,e} The serum separator tube was used to collect an initial 0.5 mL of blood, which was discarded; this was done to reduce the influence of tissue thromboplastin or other procoagulant factors that may have been introduced during initial puncture of the vessel wall, potentially altering the results of hemostatic testing. After sample collection, the tubes containing citrated blood were carefully inverted 5 times and then stored at room temperature (approx 20°C) for 30 minutes prior to TEG analysis.

TEG and other hematologic analyses—Automated platelet count,^f PCV, and total solids were determined from EDTA-anticoagulated blood within 1 hour after collection. A manual platelet count was performed when an automated count was not possible because of microscopic clumping (n = 4 samples).

Thromboelastography was performed with a commercial thromboelastograph^g in accordance with the manufacturer's recommendations. Briefly, 1 mL of citrated whole blood was added to a manufacturer-supplied vial containing a mixture of kaolin, buffered stabilizers, and a blend of phospholipids. The vial was carefully inverted 3 times to ensure adequate mixing. Thromboelastography pins and cups supplied by the manufacturer were loaded in 2 channels, and 20 μ L of calcium chloride (0.2M) was added to each cup. Then, 340 μ L of kaolin-activated citrated whole blood was added to each cup, for a total volume of 360 μ L/cup. After this, each cup was gently raised into its respective pin, and dual TEG measurements were initiated. The remaining 1.7 mL of citrated whole blood was centrifuged at $3,000 \times g$ for 10 minutes, and the plasma was stored at -80°C for future coagulation testing.

Four TEG variables were measured: R (reaction time [time to initial clot formation]), K (clotting time), α angle (measure of the rate of clot formation), and MA (related to final clot strength). The TEG assay was started exactly 30 minutes after blood collection and run at least until the final MA had been recorded.

Frozen citrated plasma samples were stored between 2 and 8 months, depending on the date of collection. The samples were then thawed and assayed on a commercial coagulation analyzer^h to determine activated partial thromboplastin time, prothrombin time, anti-thrombin concentration, and fibrinogen concentration.

Statistical analysis—The distribution of hematologic data was assessed with the Kolmogorov-Smirnov test; 95% reference intervals were calculated as mean \pm 2 SDs. Comparison of the effects of sedation on TEG variables was assessed with a paired *t* test, with values of $P < 0.05$ considered significant. Intra-assay variability was calculated on the basis of percentage deviation of duplicate TEG variables from their respective mean. All statistical analysis was performed with commercially available software.ⁱ

Results

Cats—Thirty cats (17 castrated males and 13 spayed females) were successfully enrolled in the study, with a median age of 5 years (range, 2 to 11 years). The most common breed was domestic shorthair (n = 21). Additional breeds included domestic longhair (n = 4), Siamese (2), and 1 each of Cornish Rex, Bengal, and Ragdoll. One cat was excluded because of hyperproteinemia, and 6 cats were excluded because hypertrophic cardiomyopathy was diagnosed during the echocardiographic examination. Three cats were excluded on the basis of their demeanor and the inability to obtain a clean and uninterrupted blood sample. Ten cats required sedation with butorphanol and midazolam for blood sample collection. No cats were excluded from the study on the basis of intra-assay variability.

TEG and other hematologic variables—

Determination of mean platelet count was done from 26 blood samples in which no clumping was visible. Of the 4 samples with microscopic clumping, estimated platelet counts were all within reference intervals (200,000 to 500,000 platelets/ μ L). Mean \pm SD values of hematologic variables for the included cats were as follows: PCV, $36.0 \pm 4.4\%$; total solids concentration, 7.1 ± 0.6 g/dL; platelet count, $295,000 \pm 89,000$ platelets/ μ L; prothrombin time, 9.4 ± 0.7 seconds; activated partial thromboplastin time, 14.3 ± 4.1 seconds; anti-thrombin concentration, 112.9 ± 11.1 mg/dL; and fibrinogen concentration, 197.3 ± 33.7 mg/dL. Mean \pm 2 SD values for TEG variables were as follows: R, 4.3 ± 2.4 minutes; K, 1.6 ± 1.0 minutes; α angle, $66.5 \pm 14.6^\circ$; and MA, 56.4 ± 11.2 mm.

Comparisons—Mean \pm 2 SD values for TEG variables in cats requiring sedation versus those not requiring sedation were as follows: R, 3.9 ± 1.8 minutes versus 4.5 ± 2.7 minutes, respectively ($P = 0.29$); K, 1.3 ± 0.4 minutes versus 1.8 ± 1.0 minutes, respectively ($P = 0.004$); α angle, $71.2 \pm 5.4^\circ$ versus $64.1 \pm 15.4^\circ$, respectively ($P = 0.009$); and MA, 57.9 ± 5.6 mm versus 55.7 ± 13.1 mm, respectively ($P = 0.32$).

Mean intra-assay variability for TEG variables was as follows: R, 1.8% (range, 0% to 13%); K, 4.5% (range, 0% to 10%); α angle, 2.0% (range, 0% to 17%); and MA, 1.6% (range, 0% to 6%). Mean intra-assay variability for TEG values in cats requiring sedation versus those not requiring sedation was as follows: R, 1.2% versus 3.0%, respectively ($P = 0.41$); K, 2.1% versus 3.1%, respectively ($P = 0.25$); α angle, 0.8% versus 4.0%, respectively ($P = 0.20$); and MA, 1.8% versus 1.1%, respectively ($P = 0.27$).

Discussion

Minimal intra-assay variability was identified in the present study for 4 standard TEG variables in 30 echocardiographically normal cats when a venipuncture technique designed to minimize activation of the coagulation cascade was used. This minimal variation should translate into better repeatability of assays run at different times than is possible with standard blood collection technique with a needle and syringe, and such repeatability is critical when monitoring patients with hemostatic disorders.

Intra-assay and interassay variability has been reported in values of TEG variables in cats.^{10,11,b} In 1 study,¹⁰ 2 patterns of clot retraction were evident when the LY60 values were examined. In another study,¹¹ 3 methods of TEG analysis were evaluated in 15 healthy cats, showing acceptable degrees of repeatability for R, α angle, K, and MA. Values for LY30 and LY60, both indicators of fibrinolysis, had good repeatability for kaolin- and tissue factor-activated samples but not for citrated native samples. Thromboelastography results for kaolin- and tissue factor-activated samples had better repeatability than those for citrated native samples.¹¹

One study¹⁰ in which citrated blood samples were used for TEG showed that use of citrated samples is more prone to greater intra-assay variability in cats

than are other types of blood sample treatments. All of the TEG variables evaluated in the present study had $< 20\%$ intra-assay variability. We did not evaluate LY30 and LY60 in all the cats in the present study and often stopped the TEG assay after a final MA value was reported because of the need for TEG to be performed for other hospitalized patients. Furthermore, LY30 and LY60 are not routinely evaluated in clinical human or veterinary medicine.¹⁹ We did not evaluate clot stability (a mathematical transformation of MA) because it is directly related to MA and does not provide additional information on clotting function.

Repeatability over time is expected to be important for additional TEG applications that have not been evaluated in cats such as platelet mapping, the results of which rely on accurate MA results. Platelet mapping allows evaluation of the degree of platelet inhibition in patients receiving antiplatelet medications such as acetylsalicylic acid or clopidogrel. We did not evaluate longitudinal repeatability in the present study, and it is possible that values of TEG variables could vary substantially with time because of factors such as biological variability or a cat's degree of excitement.

Mild sedation when required may help reduce any influence of excitement on results. Sedated cats in the present study had values of K and α angle that significantly differed from those of nonsedated cats, with sedated cats having a mild increase in the rapidity of clot formation. We did not compare the effects of sedation versus non-sedation on any TEG variables within the same cats; thus, it remains possible that the differences observed reflected differences between groups other than the effects of sedation. Although sedation was helpful in aiding atraumatic venipuncture and blood collection, it is unknown whether any degree of distress in these cats prior to sedation might have altered their coagulation system to the point of affecting the measured values of TEG variables. Alternatively, sedation with butorphanol and midazolam could have altered hemodynamics through a decrease in heart rate, cardiac output, and blood pressure.

Decreases in vascular shear forces and vasodilation as well as attenuation of the prothrombotic state that may occur with distress and an increase in sympathetic tone²⁰ could result in such changes as prolonged R, decreased α angle, increased K, and decreased MA, compared with values in calmer states. However, actual changes in α angle and K did not follow this pattern. No significant differences were noted in R and MA or intra-assay variability between cats that did and did not require sedation. Additionally, the observed range of R and MA values for cats requiring sedation was less than and fell within the range of those cats not requiring sedation.

The MA is arguably the most evaluated TEG variable in a clinical veterinary setting and is reflective of final clot strength; R is likely second in importance in the diagnosis of hemostatic changes because it reflects changes in the coagulation factors. The fact that neither R nor MA was significantly affected in cats that were sedated suggests that the potential effects of sedation on TEG variables in cats may not have major importance in clinical decision making. However, if one chose to compare TEG results in an individual cat over time,

then it would be important to ensure the same sedation or restraint protocol was used each time.

Results of the present study differ from those of other reported TEG reference intervals for clinically normal cats. Reference intervals for TEG variables estimated in a different study^c by means of kaolin-activated blood samples from 17 healthy cats indicate slower clot development and weaker clot strength (longer R and K and smaller α angle and MA) than was observed in the present study. Other reports^{10,11,c} of TEG reference intervals in healthy cats determined by use of kaolin or tissue factor activation or citrated native whole blood provide values closer to those observed in the present study.

Whether exclusion of cats with subclinical cardiomyopathy from the study sample considerably affected our reported reference intervals is unknown because TEG was not performed in those cats. However, results of the present study were similar to those reported for TEG variables measured in kaolin-activated blood samples from 15 clinically normal cats that were not screened echocardiographically for occult cardiomyopathy.¹¹ The reference intervals obtained in our study cannot be compared with those obtained in other studies because of differences in activators used, blood collection techniques, sample size, and exclusion criteria. For these reasons, it remains unclear whether subclinical cardiomyopathy can substantially alter TEG results in cats. Initial data analysis in a preliminary TEG study^j of hemostatic alterations in cardiomyopathic cats identified significant differences in values of coagulation variables between diseased and clinically normal cats. However, cats with subclinical cardiomyopathy were not evaluated.

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