Original Study

Thromboelastographic monitoring of the effect of unfractionated heparin in healthy dogs

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Abstract

Objective – To characterize the correlation between thromboelastography (TEG) variables using strong activators and anti-Xa (AXa) activity in healthy dogs administered subcutaneous unfractionated heparin (UFH).
Design – Prospective experimental study.
Setting – University research facility.
Animals – Eight adult random-source male dogs.
Intervention – Dogs were randomized to receive subcutaneous UFH at 200, 250, or 300 IU/kg every 8 hours for a total of 10 injections. Blood samples were collected at time 0 (preheparin) and 3, 6, and 8 hours after the 1st (Day 1) and 10th (Day 4) UFH injection. After the 8-hour blood sample was obtained on day 4, a 100 IU/kg IV bolus of UFH was administered and an additional blood sample was collected 1 hour later (hour 9). AXa activity, activated partial thromboplastin time (aPTT), and TEG (with up to 5 activators) were performed at each time point. Modes of activation for TEG included recalcified (Ca), Ca with heparinase (CaH), CaH and tissue factor 1:3600 (CTF3600H), Ca with tissue factor 1:100 (CTF100), and RapidTEG. Spearman rank correlations were calculated for each of the aforementioned parameters and the AXa activity. *P*-values were corrected for multiple comparisons with a Bonferroni correction.
Measurements and Main Results – Significant correlations were found between AXa activity and the TEG R values generated with CTF100 (R = 0.83, $P \leq 0.0001$) and RapidTEG (R = 0.90, $P < 0.0001$), as well as both forms of aPTT measurement (R = 0.86 and 0.84, $P < 0.0001$).
Conclusions – This study demonstrates that TEG variables derived using robust activation correlate with AXa activity as well as aPTT and have the potential to be used for monitoring UFH therapy in healthy dogs. Future studies are warranted to evaluate its diagnostic utility in critically ill animals.

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Abbreviations

aPTT	activated partial thromboplastin time
AXa	anti-Xa
Ca	recalcified
CaH	recalcified with heparinase
CP a PTT	clinical pathology activated thromboplastin time
CTF100	citrated tissue factor 1:100
CTF3600H	citrated tissue factor 1:3600 with heparinase
MA	maximum amplitude
POC a PTT	point-of-care activated thromboplastin time
TEG	thromboelastography
TF	tissue factor
TPP	total plasma protein
UFH	unfractionated heparin

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Introduction

Thromboembolic disease remains a major contributor to the morbidity and mortality of critically ill canine patients with various immune-mediated, endocrine and inflammatory conditions including immune-mediated hemolytic anemia (IMHA), hyperadrenocorticism, protein-losing enteropathy, and protein-losing nephropathy.[1–9] Unfractionated heparin (UFH), which requires titration, is commonly used in these veterinary patients for thromboprophylaxis, necessitating measurement of efficacy. Although the measurement of heparin concentrations for this purpose seems appealing, assays are neither readily available nor predictive of the biologic or anticoagulant effect. Numerous nonanticoagulant interactions with proteins and cells limit the percentage of heparin available for participation in anticoagulation.[10–12]

Measurement of the activity of the plasma protein Xa, which is inhibited by heparin, is currently considered a "gold standard" for monitoring and adjusting heparin use in human medicine.[10,13–16] Although the best monitoring approach is still debated in veterinary medicine, the use of Xa activity has emerged as a possible method.[17–19] For example, a recent randomized, prospective, controlled clinical trial in dogs showed an 85% reduction in 180-day mortality when UFH anticoagulant therapy was adjusted based on anti-Xa (AXa) activity versus fixed dose administration in dogs with IMHA.[18] The assay measures the amount of active Xa (i.e, not bound to antithrombin or heparin), using a fluorescent antibody. The fluorescence detected is inversely proportional to the heparin activity, which is commonly referred to as the AXa concentration.[19] Despite data supporting its use, lack of availability, as well as biological issues that may confound results,[16] make this test impractical in veterinary medicine.

The anticoagulant activity of UFH as measured by activated partial thromboplastin time (aPTT) is commonly used as a therapeutic target.[10] The goal of UFH therapy is to prolong the aPTT into a target therapeutic range (1.50–2.5 times normal baseline). Despite its ease of use, variation in reagents and instrumentation among hospitals creates significant disparities in measured aPTT in response to heparin therapy.[10,13–15] Moreover, clinically relevant correlations between aPTT and AXa activity have been inconsistently observed in human and veterinary patients.[16,17]

Thromboelastography (TEG) is a whole blood assay used to assess the viscoelastic properties of clot formation and provide quantitative measurements of clot kinetics and strength, which aid in the diagnosis of hypo- and hypercoagulable states.[20–26] A TEG instrument measures four main variables: R, alpha, K, and maximum amplitude (MA), that relate to clot initiation (R), kinetics (alpha, K), and strength (MA). This modality has been widely used in veterinary medicine to assess hemostasis.[20–22,25] Conventional coagulation tests, such as aPTT, are made to examine a specific portion of the hemostatic system, whereas, TEG is used to assess the product of the interaction of enzymatic factors and cellular components, such as red blood cells and platelets. This has the theoretical benefit of incorporating multiple variables associated with hemostasis (eg, proteases, cell counts, fibrinogen, acute phase proteins, etc), which are often dynamic during the course of an illness.

The use of TEG in monitoring UFH therapy is relatively unexplored in veterinary medicine. One previous study in dogs concluded that TEG is too sensitive to the presence of UFH, thereby limiting its use as a monitoring tool.[27] Interestingly, this study did not use an activator to stimulate clot formation, which is needed in the presence of heparin and is recommended for the performance of TEG.[28]

The goal of this study was to document the correlation between AXa activity and TEG variables using strong activators as well as aPTT in healthy dogs administered subcutaneous UFH. A strong correlation between TEG measurements and AXa activity was anticipated.

Materials and Methods

Eight random source dogs between 2 and 4 years of age including 4 intact and 4 neutered males were used for this study. Dogs were transported to an indoor canine university-owned facility where they underwent a period of adjustment and health monitoring for several weeks before experimental testing began. Bloodwork upon entry consisted of a complete blood count and biochemistry profile. The dogs initially took part in an olfaction study. Details of that study, including housing and husbandry, have already been published.[29] The median weight of the cohort was 26.2 kg (range: 24.2–28 kg). Upon subsequent entry to this study, dogs were deemed healthy based on physical examination, complete blood count,[a] Azostix,[b] and fibrinogen concentration.

Each dog was randomized, by a computer-generated spreadsheet, to receive subcutaneous unfractionated heparin (UFH)[c] at a dosage of 200, 250, or 300 IU/kg every 8 hours for 4 days. Each dog was only used once. All animal use was reviewed and approved by the Institutional Animal Care and Use Committee.

Sample collection

A modified Seldinger technique was used to place an 18 Ga, 25 cm catheter[d] into the left and then right lateral saphenous veins in each dog on days 1 and 4 after

sedation with butorphanol[e] (0.5 mg/kg, IV). As stated above, dogs were randomized to receive subcutaneous UFH at 200 (4 dogs), 250 (2 dogs), or 300 (2 dogs) IU/kg every 8 hours for a total of 10 injections. Blood samples were collected at time 0 (preheparin) and 3, 6, and 8 hours after the 1st (day 1) and 10th (day 4) UFH injection. After the 8-hour blood sample was obtained on day 4, a 100 IU/kg IV bolus of UFH was administered and an additional blood sample was collected 1 hour later (hour 9). The catheters were flushed with 3 mL of 0.9% saline after each sampling time point, and the catheter was removed at the end of each sampling day. This dosing regimen was designed to ensure subtherapeutic, therapeutic and supratherapeutic concentrations of heparin and was based upon a previous study,[27] which documented a range of AXa activity. Samples drawn on day 1 were considered likely to be subtherapeutic, those obtained on day 4 were intended to be therapeutic, and the samples obtained on day 4 with higher doses (250 or 300 IU/kg) and after an IV bolus of UFH were considered likely to be supratherapeutic based on AXa concentrations.

A total of 6.1 mL of blood was drawn from the catheter at each time point. Six milliliters of blood was removed and reserved as a purge sample. A second syringe was used to collect the 6 mL diagnostic sample, which was handed off to a second investigator, and 0.1 mL was removed using a 1 mL syringe and set aside by the first investigator. The purge sample then was then returned to the dog via the catheter. The catheter was flushed with 3 mL of 0.9% saline and clamped close to the port, prior to disconnection. The second investigator placed an 18 Ga needle on the sample syringe, and the blood was transferred into 3 citrate tubes[f] (with a final ratio of 1 part citrate to 9 parts whole blood) and one EDTA tube.[g] Citrate and EDTA tubes were each inverted by hand 5 times and then placed on a rocker. The remaining 0.5 mL of blood was discarded. The first investigator used the 0.1 mL of whole blood to immediately perform a point-of-care whole blood aPTT (POC a PTT).[h] Once the aforementioned was completed, samples were removed from the rocker and processed or allowed to sit upright, if being used for TEG. The aforementioned protocol was the same at time points including RapidTEG, except an additional 1.5 mL was taken for 1 additional citrate tube.

Analysis of blood samples

Blood samples at time 0 and hour 8 on days 1 and 4 were processed to obtain PCV total plasma protein (TPP), platelet count,[a] and fibrinogen concentration. All blood samples were processed to obtain the following information: aPTT via point-of-care[h] and bench-top analyzer,[i] AXa activity,[j] and TEG[k] using various modes of activation. A citrated whole blood sample was submitted within 10 minutes of collection for bench-top aPTT measurement through the clinical pathology laboratory (clinical pathology activated partial thromboplastin time [CP a PTT]), and a POC a PTT was performed as previously described. Citrated whole blood samples for TEG were processed after a 30-minute rest period at room temperature (approximately 23°C) according to standard technique for TEG[l] analysis. Disposable cups and pins[m] were loaded into the prewarmed channels (37°C) and 20 μL of calcium chloride[m] was added to each cup. Five TEGs were run on each sample with the following modes of activation: recalcified (Ca), Ca with heparinase[m] (CaH), CaH and citrated tissue factor[n] 1:3600 (CTF3600H), Ca with citrated tissue factor 1:100 (CTF100), and proprietary TF/kaolin mixture called RapidTEG.[m] In 4 dogs, RapidTEG was performed on samples from day 1 at time 0 and 6, and on Day 4 at time 6 and 9. This modified timing was chosen to achieve statistical power with the minimal number of samples due to the higher cost associated with the RapidTEG assay. An interim analysis at the halfway point, for dogs 1 through 4, revealed this assay to be performing well. At that point, the full complement of samples was planned for the remaining dogs. For dog 5, 6 time points out of 9 were obtained and for dogs 6–8, all 9 time points were obtained. The dog numbers at each time point are summarized in Table 1. A single operator performed all RapidTEG thromboelastograms and a second operator performed the other 4 assays. Computer randomization was used to allocate activators used to various TEG channels to prevent consistent bias. Plasma was frozen (−80°C) for batch analysis of AXa activity.[19] Platelet counts were performed using an automated analyzer.[a] Fibrinogen concentration was determined using a standard semiquantitative heat precipitation method.[30]

Statistical analysis

Spearman rank correlations were calculated for each of the TEG parameters, aPTT values, and the AXa concentrations. Current standard of care includes prolongation of aPTT by a set ratio (traditionally 1.5–2.5 times the baseline aPTT). Thus, the relationship between aPTT ratios and AXa activity was also analyzed by calculating this ratio (measured aPTT/baseline aPTT at time 0; pre-UFH administration) for every time point using each method. Bonferroni correction was applied to obtain P value cut-offs for significance, and an overall alpha rate of $P < 0.05$. P-values are presented in their raw, uncorrected form, but inference is based on corrected thresholds. Sample size calculations demonstrated that with 8 dogs, using an F distribution and a corrected alpha of 0.05, the current study had at least 80% power to detect

Table 1: Raw data for AXa, POC PTT ratio, and CP PTT ratio, and the TEG variables R, K, α, and MA for all TEGs performed classified by activator on re-calcified citrated whole blood from 8 healthy dogs administered subcutaneous UFH. Data are expressed as median and range for each sampling time point. Blood samples were collected at baseline (Time 1) before UFH, and 3 (Time 2), 6 (Time 3), and 8 (Time 4) hours after the 1st UFH injection and prior to (Time 5), as well as 3 (Time 6), 6 (Time 7), and 8 (Time 8) hours after the 10th consecutive heparin injection. An IV bolus of UFH was then administered, and a final sample taken one hour post (Time 9). The sample size (n) at each time point is expressed using the numerator to represent the number of samples that generated a measureable value, and the denominator represents the total number of samples run at that time point

		Reference Values	Time 1 (Baseline)	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7	Time 8	Time 9	P-value
AXa concentration	AXa (units/mL)		0, 0–0.1 (n = 8/8)	0.2, 0.1–0.3 (n = 8/8)	0.2, 0.1–0.3 (n = 8/8)	0.2, 0.1–0.3 (n = 8/8)	0.2, 0.1–0.8 (n = 8/8)	0.6, 0.1–0.9 (n = 8/8)	0.5, 0.1–0.9 (n = 8/8)	0.3, 0.1–0.7 (n = 8/8)	1.1, 0.6–1.3 (n = 7/7)	<0.001*
aPTT ratio	POC PTT ratio	1.0	(n = 8/8)	1.1, 1–1.2 (n = 8/8)	1.1, 0.9–1.3 (n = 8/8)	1.1, 0.99–1.2 (n = 8/8)	1.2, 1–1.6 (n = 8/8)	1.4, 1–2.2 (n = 8/8)	1.2, 1–1.9 (n = 8/8)	1.2, 0.8–1.6 (n = 8/8)	2, 1.7–2.4 (n = 7/7)	<0.001*
	CP PTT ratio	1	(n = 8/8)	1.2, 1.0–1.3 (n = 8/8)	1.2, 1.1–1.4 (n = 8/8)	1.1, 1.1–1.4 (n = 8/8)	1.2, 1.1–2.2 (n = 8/8)	1.8, 1.1–4.3 (n = 8/8)	1.5, 1.1–3.7 (n = 8/8)	1.3, 1.1–2.5 (n = 8/8)	2.9, 2–8 (n = 7/7)	<0.001*
CN	R (min)	4.1, 2.2–8.4 (n = 17)	4.8, 3.7–7.8 (n = 8/8)	120, 5.7–120 (n = 8/8)	89.1, 19.3–120 (n = 8/8)	61.7, 9.2–120 (n = 8/8)	120, 11.1–120 (n = 8/8)	120, 44.8–120 (n = 8/8)	120, 28.3–120 (n = 8/8)	120, 21–120 (n = 8/8)	120 (n = 1/8)	<0.001*
	K (min)	2.3, 1.3–3.4 (n = 17)	1.9, 1.1–3.4 (n = 8/8)	7.0, 2.7–11.2 (n = 2/8)	10.9 (n = 1/8)	12.4, 3.9–20.8 (n = 2/8)	27.7, 8.4–47 (n = 2/8)	(n = 0/8)	39.3 (n = 1/8)	25.1 (n = 1/8)	(n = 0/8)	ns
	α (degrees)	59.5, 48.5–71.1 (n = 17)	64.6, 55.4–67.8 (n = 8/8)	19.3, 4–56 (n = 3/8)	2.8, 0.8–19 (n = 4/8)	1.9, 0.7–43.7 (n = 6/8)	5.2, 2.6–25.5 (n = 3/8)	1.5 (n = 1/8)	4.6, 3.3–5.9 (n = 2/8)	7.2, 5–9.4 (n = 2/8)	(n = 0/8)	ns
	MA (mm)	55.5, 49.2–63.9 (n = 17)	51.8, 42.1–65.3 (n = 8/8)	37.1, 2.9–44.4 (n = 3/8)	7.4, 2.3–39.4 (n = 4/8)	4.5, 2.3–49 (n = 6/8)	31.6, 2.8–40.3 (n = 3/8)	2.4 (n = 1/8)	27.0, 9–45 (n = 2/8)	16.9, 10.5–23.3 (n = 2/8)	(n = 0/8)	ns
CNH	R (min)	NA	5.2, 3.7–7 (n = 8/8)	9.7, 6.5–13.7 (n = 6/8)	9.6, 6.1–17.3 (n = 8/8)	9.4, 5.8–15.8 (n = 8/8)	8.4, 5.6–12.6 (n = 8/8)	9.9, 7.2–12.7 (n = 8/8)	10.2, 8.2–13.8 (n = 6/8)	8.1, 4.8–13.8 (n = 8/8)	15.7, 9.2–16.1 (n = 7/7)	0.001*
	K (min)	NA	2.1, 1.3–3.8 (n = 8/8)	3.5, 1.8–5.3 (n = 6/8)	3.9, 1.8–11.8 (n = 8/8)	3.7, 2.2–8.1 (n = 8/8)	3.0, 1.2–5.8 (n = 8/8)	4.1, 2.2–6.2 (n = 8/8)	3.8, 2.4–6.4 (n = 6/8)	2.8, 1.5–6.5 (n = 8/8)	7.2, 3.5–10.3 (n = 7/7)	0.007
	α (degrees)	NA	62.3, 47.5–70.7 (n = 8/8)	46.4, 34.4–65.4 (n = 6/8)	39.9, 18.8–65.8 (n = 8/8)	46.4, 37.8–60.4 (n = 8/8)	49.8, 33.5–70.9 (n = 8/8)	41.0, 30.3–60.4 (n = 8/8)	43.8, 31.7–56.8 (n = 6/8)	52, 27.6–69.5 (n = 8/8)	26.6, 20–46.5 (n = 7/7)	0.005
	MA (mm)	NA	51, 43.2–56.5 (n = 8/8)	49.7, 45.4–58.5 (n = 6/8)	49.8, 39.6–57.7 (n = 8/8)	49.1, 41.6–58.4 (n = 8/8)	54.5, 40.5–60.6 (n = 8/8)	50.6, 39.9–61.7 (n = 8/8)	51.8, 37.2–59.1 (n = 6/8)	56, 39.9–62.4 (n = 8/8)	46.6, 37.3–54.2 (n = 7/7)	0.866

(Continued)

Table 1: (Continued)

		Reference Values	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7	Time 8	Time 9	P-value
CTF34H	R (min)	NA	2.0 1.7–3.2 (n = 8/8)	2.2 1.8–3.9 (n = 8/8)	2.4 1.8–2.8 (n = 8/8)	2.2 1.9–3.4 (n = 8/8)	2.5 1.8–4.2 (n = 8/8)	2.6 1.9–4.2 (n = 8/8)	2.8 2.2–6.2 (n = 8/8)	2.6 1.8–4.2 (n = 8/8)	3.2 2.5–4.6 (n = 7/7)	0.001*
	K (min)	NA	1.8 1.2–2.9 (n = 8/8)	2.0 1.2–2.9 (n = 8/8)	2.0 1.2–2.8 (n = 8/8)	1.75 1.4–3.1 (n = 8/8)	2.4 1.2–5.1 (n = 8/8)	2.2 1.1–6.9 (n = 8/8)	2.6 1.6–5.1 (n = 8/8)	2.1 1.3–4.8 (n = 8/8)	2.8 1.6–2.9 (n = 7/7)	0.092
	α (degrees)	NA	65.8 52–73.6 (n = 8/8)	62.9 53.2–73.3 (n = 8/8)	62 54.1–71.7 (n = 8/8)	63.4 50.7–68.6 (n = 8/8)	58.1 36.2–72.7 (n = 8/8)	58.6 30.2–72.4 (n = 8/8)	54.7 36.3–65.9 (n = 8/8)	61.3 37.2–71.2 (n = 8/8)	54.8 51–67.8 (n = 7/7)	0.081
	MA (mm)	NA	50.9 45.1–66 (n = 8/8)	49.9 44.4–62.5 (n = 8/8)	49.6 46.6–60.6 (n = 8/8)	51.9 38.3–61.6 (n = 8/8)	48.3 45.9–64 (n = 8/8)	49.6 35–65.5 (n = 8/8)	53 40.1–61.1 (n = 8/8)	50.7 43–60.6 (n = 8/8)	54.3 42.5–60.8 (n = 7/7)	0.687
CTF1	R (min)	0.6 0.2–1.6 (n = 20)	0.5 0.2–1 (n = 8/8)	0.8 0.7–1.3 (n = 8/8)	1.0 0.3–1.3 (n = 8/8)	0.8 0.4–1.4 (n = 8/8)	1.3 0.4–2.5 (n = 8/8)	1.8 0.7–8.8 (n = 8/8)	1.8 0.8–6.2 (n = 8/8)	1.4 0.5–3.7 (n = 8/8)	6.2 2.7–64.9 (n = 7/7)	<0.001*
	K (min)	2.3 0.8–5.5 (n = 20)	2.3 1.2–3 (n = 8/8)	2.3 1.2–3.2 (n = 8/8)	2.5 0.8–3.6 (n = 8/8)	2.1 1–3.3 (n = 8/8)	2.6 1–4.6 (n = 8/8)	2.5 0.9–3.8 (n = 7/8)	2.1 1.2–3.3 (n = 8/8)	2.7 1.2–8.3 (n = 8/8)	9.3 2.5–24.2 (n = 4/7)	0.057
	α (degrees)	66.7 49.9–81.7 (n = 20)	65.3 57–74.7 (n = 8/8)	66.5 54.6–75.8 (n = 8/8)	61.7 54.3–75.3 (n = 8/8)	66.6 57.8–77.9 (n = 8/8)	62.2 44.9–76.9 (n = 8/8)	59.7 7.1–77.8 (n = 8/8)	61.9 10.7–75.2 (n = 8/8)	59.3 34.6–74.8 (n = 8/8)	11.4 0.6–57.4 (n = 7/7)	0.003*
	MA (mm)	41.8 36.5–53.6 (n = 20)	44.7 36.5–53.4 (n = 8/8)	44.9 36.5–47 (n = 8/8)	42.8 36.7–68.4 (n = 8/8)	46.1 36.2–57.1 (n = 8/8)	47 37.1–60.7 (n = 8/8)	45.9 10.4–59 (n = 8/8)	43.5 19.2–59.2 (n = 8/8)	44.1 30.1–57.7 (n = 8/8)	23 2.3–53.2 (n = 7/7)	0.162
RapidTEG	R (min)	0.5 0.3–1.2 (n = 47)	0.5 0.3–0.8 (n = 8/8)	1 0.7–1.1 (n = 3/3)	0.6 0.4–1.1 (n = 7/7)	0.8 0.5–0.8 (n = 3/3)	1.1 0.8–1.8 (n = 4/4)	1.7 1.2–2.2 (n = 4/4)	1.2 0.3–2.2 (n = 8/8)	1.4 1–1.7 (n = 4/4)	1.9 1.7–3.8 (n = 7/7)	<0.001*
	K (min)	2.7 0.8–3.7 (n = 47)	2.3 1.2–3.8 (n = 8/8)	1.8 1.4–2.7 (n = 3/3)	1.7 0.9–2.2 (n = 7/7)	1.8 1.7–1.9 (n = 3/3)	2 1.5–2.7 (n = 4/4)	2.1 1.5–2.7 (n = 4/4)	1.9 1–3.2 (n = 8/8)	2 1.3–3.1 (n = 4/4)	1.8 1.3–3.3 (n = 7/7)	0.838
	α (degrees)	63.8 52.5–76.8 (n = 47)	62.7 56.4–77.2 (n = 8/8)	65.2 59.5–70.3 (n = 3/3)	67.9 63.6–78 (n = 7/7)	67.4 66.6–68.4 (n = 3/3)	63.1 58.5–69.8 (n = 4/4)	61.4 55.4–68 (n = 4/4)	66.4 51.9–76.8 (n = 8/8)	63.6 53.6–70 (n = 4/4)	62 48.2–69.8 (n = 7/7)	0.283
	MA (mm)	51.6 42.0–66.3 (n = 47)	50.1 48.4–61.8 (n = 8/8)	52.9 50.6–54.2 (n = 3/3)	54.8 46–67.9 (n = 7/7)	57.7 56.8–62.1 (n = 3/3)	52.6 48.3–64.1 (n = 4/4)	59.1 52.6–64.1 (n = 4/4)	58.1 48.1–66.8 (n = 8/8)	58.4 48.5–68.3 (n = 4/4)	56.9 54.9–67.3 (n = 7/7)	0.019

If a flatline tracing was obtained, R values were truncated and recorded as 120 minutes, and all other values for that tracing were considered unmeasureable. Institutional reference ranges, when available, are included for each of the TEG methodologies. Due to the multiple comparisons provided, only statistically significant trends in each row are noted. After Bonferroni correction, p-values less than 0.0025 are deemed significant (∗). Ins signifies Insufficient data to be analyzed.

Table 2: Spearman's correlation coefficients between antiXa (AXa) activity and thromboelastography (TEG) variables obtained using one of the following 5 activators in citrated whole blood from 8 healthy dogs administered subcutaneous unfractionated heparin (UFH): recalcified (Ca), Ca with heparinase (CaH), CaH and tissue factor 1:3600 (CTF3600H), Ca with tissue factor 1:100 (CTF100), and RapidTEG

TEG variable	Ca	CaH	CTF3600H	CTF100	RapidTEG
R (min)	0.66	0.66**	0.60**	0.83**	0.90**
K (min)	0.76*	0.64**	0.37*	0.33	−0.01
Angle (deg)	−0.71*	−0.63**	−0.36*	−0.51***	−0.25
MA (mm)	−0.68*	−0.35	−0.21	−0.20	0.32

*P-value 0.005–0.05.
**P-value 0.001–0.004.
***P-value < 0.001.

correlations between Xa activity and TEG parameters of at least ±0.78. Sign tests and mixed model regression analyses were then used to analyze the data, testing both for associations among variables and to predict and characterize therapeutic ranges. The linear relationship between each variable (TEG, aPTT, and aPTT ratios) and AXa concentrations was plotted using the least-squares regression technique with a line of best fit, according to recommendations for establishing therapeutic ranges for heparin therapy in people.[14] The therapeutic range was then determined using the ordinate values on the least squares regression line that corresponds to plasma AXa activity of 0.3 to 0.7 IU/mL.[16] To test for trends in PCV, TPP, fibrinogen, and platelet concentrations over time, a nonparametric Mann-Kendall test was performed. To test for trends in raw values obtained with the 5 different activators, a nonparametic Mann-Kendall test was again performed. The study was not powered with this as a primary objective, thus after correcting for all the comparisons, only P values of <0.0025 were deemed significant. All statistical analyses were performed using commercial statistical software.[n,o]

Results

A summary of all data for each test and activator are included in Table 1. The Spearman's correlations (ρ) for each of the aforementioned TEG variables and AXa activity are summarized in Table 2. The data for the TEG-derived R variables, having strongest correlations (ρ), with associated AXa activity are presented in Figures 1 and 2.

The Spearman's correlation between AXa activity and the CP a PTT was 0.84 ($P < 0.001$). The Spearman's correlation between AXa activity and the POC a PTT was 0.86 ($P < 0.001$). The Spearman's correlation between AXa activity and CP a PTT ratio was 0.85 ($P < 0.001$) and POC a PTT ratio was 0.83 ($P < 0.001$).

The linear relationship between variables with strong correlations to AXa concentrations was plotted using the least-squares regression technique with a line of best fit. The therapeutic ranges for CTF100-derived TEG variable R, RapidTEG variable R, POC a PTT, and CP a PTT ratios were then determined using the ordinate values on the regression line that correspond to plasma AXa activity of 0.3–0.7 IU/mL. For the POC a PTT measurement, the therapeutic ratio was determined to be 1.2–1.6 times the baseline aPTT. For the CP a PTT measurement, the therapeutic ratio was determined to be 1.5–2.6 times the baseline aPTT value. The therapeutic R value derived via CTF100 activation was 1.5–3.7 minutes. The therapeutic R value derived via RapidTEG activation was 0.9–1.8 minutes.

Mean baseline and final PCV were 46 ± 4.7% and 42 ± 3.3%, respectively. The mean baseline and final TPP were 60 ± 5.0 g/L and 62 ± 3.9 g/L, respectively. The mean baseline and final platelet counts were 233, 222 ± 42,000/μL and 228, 333 ± 45,000/μL, respectively. The mean baseline and final fibrinogen values were 3.91 ± 1.47 μmol/L and 3.58 ± 1.29 μmol/L . The results of the Mann–Kendall tests reveal that all the variables were significantly different over time ($P < 0.001$).

Discussion

In this study, healthy research dogs were administered subcutaneous UFH and the statistical correlations between AXa activity and TEG variables and aPTT measurements, via 2 different methodologies, were determined. Strong statistically significant correlations were observed between AXa activity and values obtained via TEG activated with CTF100 and RapidTEG as well as both forms of aPTT measurement.

Unfractionated heparin is inexpensive, reversible, can be administered by multiple routes, and is likely to remain one of the most widely used anticoagulants in veterinary medicine.[10,11] UFH molecules range in size from 3,000 to 30,000 Daltons, which impacts its availability and ability to bind various coagulation proteases. UFH is also cleared by 2 mechanisms, a high-affinity saturable clearance by reticuloendothelial and endothelial cells and a nonsaturable renal route. The contribution of each varies by the dosage and route of administration. Heparin is extensively protein bound (eg, fibrinogen, lipoproteins) and also binds to macrophages, platelets, and endothelial cells.[10–12] All of these factors contribute to significant variability in the anticoagulant effect of UFH, reinforcing the need for accurate and available methods of monitoring for this widely used medication.
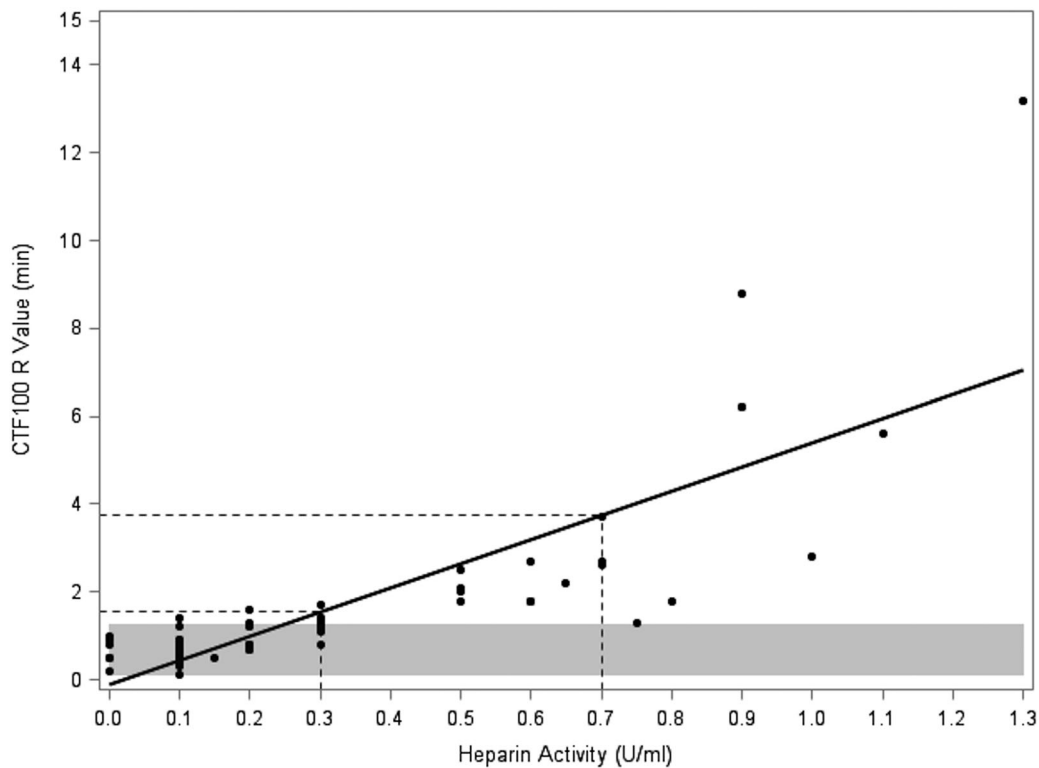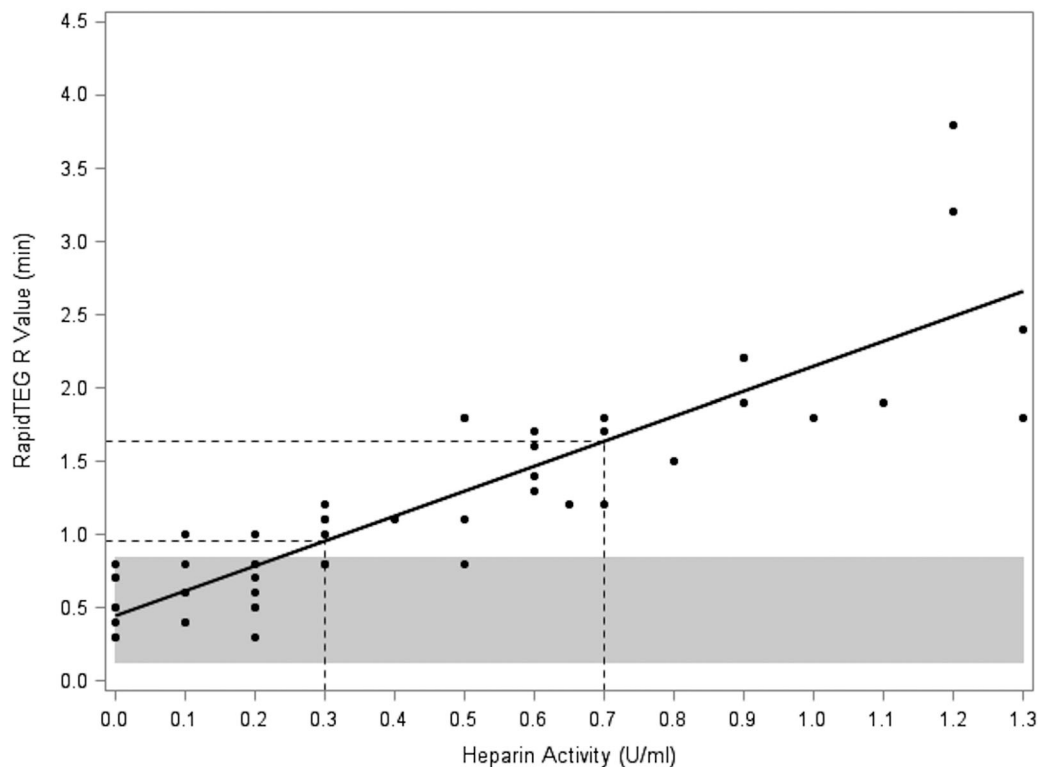
Figure 1: Scatter plot showing the relationship between anti-Xa (AXa) activity (heparin activity) and the thromboelastography variable R obtained with recalcified citrated whole blood, from 8 healthy dogs administered subcutaneous unfractionated heparin (UFH), activated with tissue factor 1:100 (CTF100) plotted using the least-squares regression technique with a line of best fit. The dotted lines represent the boundaries of therapeutic AXa activity for people. The shaded horizontal area denotes the institutional reference range for R values derived with CTF100 activation in dogs. Note: two outliers have been removed from the figure for scaling purposes. The Y and X ordinates for these outliers are 64.9; 1.3 and 24.5; 1.2.

In this study, when aPTT measured via 2 different methods (CP PTT and POC PTT) was compared to anti-Xa activity, good correlations were obtained for both aPTT tests; however, the therapeutic ranges generated in this study for aPTT measurement varied significantly depending on the aPTT methodology that was used, as has been previously demonstrated.[17] This difference in the raw therapeutic aPTT values was not unexpected. For this reason, aPTT ratios are often used in an effort to "normalize" the value relative to the patient's baseline; however, a marked difference persisted in the ranges for therapeutic aPTT ratios (measured aPTT/baseline aPTT). The American College of Chest Physicians[10] and the College of American Pathologists[13] recommend aPTT be used for monitoring UFH only after establishing an institutional nomogram via calibration using that institution's reagents and instrumentation to establish a target based on correlation with therapeutic heparin concentrations, as measured by AXa activity. Our study results suggest that veterinary laboratories should adopt human guidelines,[10,13] suggesting that aPTT measurement only be used for heparin monitoring after an institu-

tional nomogram is established using anti-Xa activity as the gold standard.

Despite being considered the "gold standard," several drawbacks remain important with respect to the measurement of AXa activity. Because AXa is an amidolytic chromogenic assay, its measurement can be influenced by anything that imparts color/pigment to the serum, including bilirubin or hemoglobin, thus confounding test interpretation in patients with hemolytic or hepatobiliary disease.[16] Although some degree of automation has occurred, instruments measuring Xa activity are only available in large diagnostic laboratories, which are not readily available to most veterinarians. Overall, the assay remains complex, with inherent background activity in the dog,[19] and is not practical without running a large number of samples, to keep the cost of each individual test in a reasonable range, making its use unlikely to become widespread or available cage side.

TEG is relatively easy to perform, financially feasible, can be performed at the point of care, in the intensive care unit or in-house laboratory, and has the benefit of being a global assessment of hemostasis. Investigators have

Figure 2: Scatter plot showing the relationship between anti-Xa activity (heparin activity) and the thromboelastography variable R obtained with recalcified citrated whole blood, from 8 healthy dogs administered subcutaneous UFH, activated with a proprietary mixture of tissue factor and kaolin (RapidTEG) plotted using the least-squares regression technique with a line of best fit. The dotted lines represent the boundaries of therapeutic AXa activity for people. The shaded horizontal area denotes the institutional reference range for R values derived with RapidTEG activation in dogs.

shown that viscoelastic testing is affected by both the cellular, such as platelet and erythrocytes, and plasmatic, such as fibrinogen and proteases, portions of blood.[31] It is arguably of benefit to have all of these factors be taken into account with one monitoring assay. One must also consider the effect of anemia on viscoelastic testing, which continues to be debated as to whether it is real or artifactual.[32,33] The effect of anemia in this study is discussed in a subsequent paragraph and is relevant to consider given that a major indication for heparin in veterinary medicine is the diagnosis of immune-mediated hemolytic anemia. Regardless, how well all of this translates into clinical practice remains to be seen. The results of this study show that TEG, with robust activation, correlates with heparin activity in healthy dogs that were assessed using AXa activity. Our results confirm a previous study[27] showing that TEG is sensitive to the presence of heparin, and when performed without any or weaker activation, such as with Ca, CaH, or TF3600H, it does not correlate well with anti-Xa activity. Given that thrombosis occurs in animals with hypercoagulable states, even in the face of heparin therapy, it is reason-

able to hypothesize that strong activation may mimic what is occurring biologically in critically ill patients, or at least in certain microenvironments. For the 5 TEG protocols that were tested in the present study, the strongest correlations were found between anti-Xa activity and the TEG-derived R variable obtained via activation with CTF100 and RapidTEG. These results are in agreement with a recent study that showed a good correlation between Sonoclot Activated Clotting Time and both AXa and aPTT.[34] Other standard variables, including MA, K, and alpha correlated poorly with anti-Xa activity and all activators used. These variables are reflective of the kinetics and strength of fibrin and platelet interactions, which may be less sensitive or less predictable in response to UFH. The TEG variable R is a measure of clot initiation, representing the start of fibrin formation, and is the least affected by other cellular components, such as red blood cells.[31] Thus, concerns regarding effects of anemia on TEG are minimized when R is used. It is logical that TEG performs similarly to aPTT as it is mimicking aPTT, from the standpoint of measuring fibrin formation. However, we propose that

an additional benefit with TEG would be the use of TF as part of the activation, which represents a more biological first step in the initiation of clot formation. One may also be able to appreciate trends over time when monitoring a patient with regard to other variables, such as MA.

RapidTEG is a dual-activated TEG that uses native (whole blood) or citrated and recalcified blood along with a proprietary combination of both kaolin and tissue-factor to more quickly produce a TEG tracing (generally 10 minutes faster than traditional TEG assays).[35,36] Its use in human medicine is primarily in the setting of trauma or cardiac surgery where rapid results are required.[36–38] The information derived from a RapidTEG tracing has also been correlated with hypercoagulability and the incidence of thromboembolism in people.[38–41] To our knowledge, this is the first veterinary study to investigate the use of RapidTEG in dogs. In the setting of UFH therapy, a robust assay is required, and RapidTEG is the most robust assay that is commercially available, making it uniquely suited to this application.

There are several limitations to this study. It was performed in healthy research dogs and is not reflective of the hemostatic changes that are expected in inflammatory or critically ill patients. Also, RapidTEGs were not collected at all time points. Although the overall RapidTEG sample sizes were small, they were sufficient based on power analysis to detect a correlation with AXa activity. However, it must be emphasized that clinical trials are needed to assess the clinical utility of these results. These dogs were all treated with subcutaneous UFH, while the patient population that is most frequently targeted for UFH usage in the hospital setting may receive IV heparin therapy. This drawback is ameliorated by the fact that the correlation is based on the measurement of anti-Xa activity, which is independent of route as this was not a pharmacokinetic study. Also, the choice of a gold standard of AXa activity in dogs is based on data in people and other species.[10–13,16] Although widely accepted, it is unknown if this is truly the superior target or even the correct therapeutic range for dogs. Furthermore, variation exists between laboratories and instrumentation for this assay in people as well, making a universal gold standard therapeutic target difficult to achieve.[42] One may also question the impact of the difference found in packed cell volume, fibrinogen, and platelet concentration over time. The authors hypothesize that these changes were secondary to sampling over time and repetitive flushing. Although statistically significant, these values are within institutional reference ranges and are not deemed clinically significant. Nonetheless, given that the clinically relevant finding in this study is the association of the TEG variable R with AXa, only the impact on the R variable needs to be discussed. In a study assessing effects of hemostatic variables on thromboelastometry (TEM), a viscoelastic test similar to TEG, in healthy dogs, no association was found between fibrinogen and clotting time (TEM equivalent of the TEG-derived R value). The Spearman's correlation between TF-activated TEM-derived clotting time and hematocrit or platelet concentration was also weak ($\rho = 0.3$, $P = 0.01$ and $\rho = 0.29$, $P = 0.01$, respectively).[31] Furthermore, the observed effect of a drop in hematocrit on fibrin initiation in thromboelastometry and TEG (clotting time and R value, respectively) is in the opposite direction to that observed in this study. Finally, one may question the activators chosen. This was based on unpublished pilot data using citrated whole blood samples spiked with heparin to varying concentrations.[p,q] Calcium was deemed our positive control, and the other activators were chosen based on good to excellent correlations obtained by in vitro methods. In addition to the activators chosen for this study, kaolin, kaolin with heparinase, citrated TF at 1:3600, and citrated TF at 1:50,000 were evaluated, but poor correlations with known AXa activity were obtained. Interestingly, using in vitro methodology, CTF100 and CTF3600H were superior and correlations with MA were superior to R. Again, this was underpowered preliminary pilot data. Yet, this is markedly different than what was demonstrated above with ex vivo methodology. This is likely due to the many factors put forth at the beginning of this discussion with regard to the significant variability in the anticoagulant effect of UFH in a patient. Last, it is also worthwhile to note that significant changes exist in samples with therapeutic AXa activity and heparinase coated cups (Table 1). Heparinase coating does not obviate all of the effects of heparin. With regard the Table 1, the authors want to again emphasize that the study was not powered with comparison of these values as the primary objective. As such, after correcting for multiple comparisons, only P values of less than 0.0025 are deemed significant. These data should be used for the appreciation of trends only.

In conclusion, this is the first veterinary study that documented the potential ability to monitor UFH therapy with TEG. Strong activation of TEG is required to obtain correlations between TEG variables and anti-Xa activity. This is also the first veterinary study detailing the use of RapidTEG in dogs. Lastly, while aPTT remains the "silver standard" for monitoring UFH therapy, CTF100 activated TEG and RapidTEG correlated as well as aPTT with anti-Xa activity. It is unknown at this time which assay will be superior in critically ill or inflammatory patients. A study is currently in progress to compare TEG with aPTT measurement for monitoring UFH therapy in critically ill canine patients.

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Acknowledgments

Footnotes

[a] Siemens Advia 120 automated hematology analyzer, Siemens Diagnostics, Deerfield, IL.
[b] Azostix, Seimens, Tarrytown, NY.
[c] Heparin, Hospira, Lake Forest, IL.
[d] Mila International Inc, Erlanger, KY.
[e] Torbugesic, Fort Dodge, New York, NY.
[f] SARSTEDT Monovette Coagulation 9 NC/1.4 mL syringes, Sarstedt Inc, Newton, NC.
[g] SARSTEDT Monovette EDTA KE/1.2 mL syringes, Sarstedt Inc.
[h] SCA2000 Coag DX Analyser, Idexx, Westbrook, ME.
[i] Satellite hemostasis analyzer, Diagnostica Stago Inc, Parsippany, NJ.
[j] Comparative Coagulation Lab, Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine, Ithaca, NY.
[k] TEG 5000 Haemostasis Analyzer, Haemoscope Corporation, Braintree, MA.
[l] TEG 5000 User's Manual, Haemoscope Corporation.
[m] Disposable TEG cups and pins, 0.2 M calcium chloride, proprietary tissue factor/kaolin mixture (RapidTEG Reagent), Disposable cups and pins with heparinase, Haemonetics, Niles, IL.
[n] Dade Innovin, Siemens Healthcare Diagnostics Inc, Newark, DE.
[o] SAS version 9. 2004. SAS Institute Inc, Cary, NC.
[p] StataCorp. 2009. Stata Statistical Software, Release 11, StataCorp LP, College Station, TX.
[q] Hanel RM. Unpublished observation, North Carolina State University, Raleigh, NC 2014.

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