**Feline aortic thromboembolism with and without congestive heart failure did not exhibit hypercoagulability using a novel viscoelastic coagulation monitor**

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OBJECTIVE

To demonstrate hypercoagulability with a benchtop viscoelastic monitor in cats with congestive heart failure (CHF) and/or aortic thromboembolism (ATE) compared to controls.

METHODS

97 cats were enrolled throughout this prospective observational cohort study from September 2022 through October 2023. Cats were grouped by diagnosis of CHF, ATE, ATE plus CHF, or controls. Enrollment required diagnosis of heart disease and no previous antithrombotic therapy. The results of viscoelastic testing with the benchtop viscoelastic coagulation monitor (VCM Vet [VCM]; Entegrion) were compared between groups using factorial analysis of variance.

RESULTS

Cats with heart disease had significantly higher clot times when compared to controls (control: mean, 285.3 [SD, 172.6]; CHF: mean, 391.5 [SD, 109.2]; and ATE: mean, 368.6 [SD, 232.6]). Heart disease cats were noted to have significantly lower 45-minute lysis index values (control: median, 100 [range, 93 to 100]; CHF: median, 99 [range, 89 to 100]; ATE: median, 98 [range, 88 to 100]; and ATE plus CHF: range, 98 [91 to 100]). Age was a covariate to this variable, and when applied to analysis, statistical significance was lost. No significant difference in any other variables were noted.

CLINICAL RELEVANCE

The hypercoagulability of ATE and CHF cats was not detected by the VCM. Further research with other coagulation monitors is required in this population.

Keywords: viscoelastic testing, hypercoagulability, feline aortic thromboembolism, feline cardiomyopathy, hyperfibrinolysis

Feline aortic thromboembolism (ATE) is a serious cause of morbidity and mortality in cats.\(^1\,^2\) ATE is commonly associated with feline heart disease, particularly hypertrophic cardiomyopathy (HCM). HCM is the most commonly documented feline cardiomyopathy, affecting 14.7% of the feline population, and ATE has a reported prevalence of 11.3% among cats with HCM.\(^3\,^4\) As it is the most common feline cardiomyopathy, the majority of research on coagulation in feline heart disease is on HCM patients. ATE has been less commonly reported secondary to other cardiomyopathy phenotypes (restrictive cardiomyopathy, dilated cardiomyopathy, nonspecific cardiomyopathy) as well as rarely being associated with neoplasia, hyperthyroidism, transient myocardial thickening, or severe systemic inflammation.\(^2\,^5\,^6\)

The lower ATE incidence in other cardiomyopathies is likely due to being less common when compared to HCM rather than true reduced risk. These thromboemboli (TE) typically lodge in the aortic trifurcation or, less commonly, in the right subclavian artery, causing ischemia, severe pain, cold extremities, and paresis of the affected limb.\(^1\,^7\) Thrombi have also been detected within the mesenteric vessels, kidneys, brain, and lungs.\(^8\,^9\) Due to the high degree of morbidity associated, there is a high mortality rate of up to 55.9% at 7 days and a reported euthanasia rate of up to 90%.\(^1\,^5\) For those that do survive, there...
is a reported 50% recurrence rate\textsuperscript{1,2,10} and an average time to recurrence or death of 11.5 months.\textsuperscript{11} Up to 88% of cats presenting with ATE will not have previously diagnosed cardiomyopathy.\textsuperscript{1} Due to the severity of this syndrome, identification of at-risk patients for thromboprophylaxis is an important area of developing research.

It is hypothesized that ATE in cats originates within the left atrium and later becomes dislodged to embolize at distant sites, resulting in the clinical signs of ATE. The formation of a TE is conventionally attributed to 1 or more of the components of the Virchow triad (blood flow stasis, endothelial injury, and hypercoagulability) being present.\textsuperscript{12} Abnormal or static blood flow has been associated with TE in both feline and human patients.\textsuperscript{4,15-17} The presence of spontaneous echocardiographic (SEC) and degree of left atrial (LA) enlargement and LA systolic dysfunction have all been demonstrated to be risk factors for the development of ATE.\textsuperscript{14,16,17} Reduction in LA function results in blood flow stasis and is in turn associated with the formation of SEC and/or intracardiac thrombus formation within the LA appendage.\textsuperscript{2} SEC is thought to occur secondary to static blood, resulting in either the formation of red blood cell fibrinogen aggregates\textsuperscript{14,18} or the formation of activated platelet-leukocyte aggregates.\textsuperscript{19}

Endothelial injury has been previously documented on necropsy examination of the hearts of cats with HCM.\textsuperscript{20-23} It has also been reported that during endothelial injury, there is a switch from the release of anticoagulant molecules, such as nitric oxide, prostaglandin I\textsubscript{2}, and thrombomodulin, to the release of procoagulants, such as von Willebrand factor (vWF) and tissue factor.\textsuperscript{2,12,14,24,25} Cats with ATE have been shown to have increased plasma vWF concentrations\textsuperscript{12} as well as an abundance of vWF detected within intracardiac thrombi.\textsuperscript{26}

There have been several ex vivo studies\textsuperscript{27} that have shown biomarkers of hypercoagulability in cats with HCM. Increased platelet microparticles and P-selectin expression was noted to correlate directly with heart disease severity. The platelets of HCM cats were also noted to be hyperresponsive, or “primed,” when compared to cats without heart disease.\textsuperscript{28,29} Cats with HCM have been shown to have deranged secondary hemostatic biomarkers, with elevated thrombin-antithrombin (TAT) complexes, fibrin degradation products (FPD), and D-dimers all being documented.\textsuperscript{12,30,31} More recently, the concept of immunothrombosis and immune-mediated hypercoagulability has come to the forefront of research surrounding thromboembolic disease.\textsuperscript{32} Neutrophils have been shown to be important in immunothrombosis, primarily through the release of neutrophil extracellular traps (NETs).\textsuperscript{35-37} These NETs are made up primarily of antimicrobial granular proteins, cell-free DNA, and citrullinated histones and are intended to trap circulating pathogens; however, they are also potently procoagulant in nature.\textsuperscript{2,34,35} Indeed, important components of NETs (cell-free DNA and citrullinated histones) have been found to be elevated in feline HCM and ATE patients, and NETs have been documented as dynamic structural components of thrombi in cats with ATE.\textsuperscript{2,34,35}

To date, there have been no published studies showing hypercoagulability using viscoelastic monitors in cats with HCM or ATE. An unpublished abstract\textsuperscript{36} was presented at the 2008 American College of Veterinary Internal Medicine forum, evaluating feline cardiomyopathy and aortic thromboembolism using thromboelastography (TEG). This demonstrated a statistical difference between ATE cats and reference ranges. Although previous\textsuperscript{12,30,31} studies have shown biomarker evidence of hypercoagulability in HCM cats, to date there have been none empirically evaluating evidence of viscoelastic hypercoagulability in these patients.

The novel benchtop viscoelastic monitor employed in this study was the viscoelastic coagulation monitor (VCM-Vet [VCM]; Entegrion). This test evaluates the entire hemostatic system from time to initial clot formation, clot kinetics, total clot strength, and rate of fibrinolysis.\textsuperscript{37} The VCM unit requires 340 μL of fresh whole blood, which must be run within 4 minutes of collection per manufacturer recommendations.\textsuperscript{37} This sample is introduced into a single-use cassette, where it is taken up by capillary action and fed into the active testing portion of the cassette. This is made up of 2 glass plates located within the cartridge, which is inserted into the benchtop unit. One of the glass plates is moved rhythmically by a motor, and the other is free standing.\textsuperscript{37} The blood undergoes contact activation by the glass, and as the blood coagulates, the plates begin to adhere to each other.\textsuperscript{37} The kinetics and rate of the developing clot are transmitted internally and displayed on the monitor as both a trace and series of values.\textsuperscript{37} This testing modality has been validated in a variety of healthy veterinary species, but there is limited data on animals with clinical disease.\textsuperscript{37}

We hypothesized that cats with clinical ATE or congested heart failure (CHF) would be detectably hypercoagulable when compared to healthy controls using the VCM. Our secondary hypothesis was that cats with HCM with or without CHF would be detectably hypercoagulable when compared to healthy controls using the VCM. We also compared the VCM values between the different disease state groups to assess any significant differences.

**Methods**

This prospective observational cohort study took place from September 2022 through December 2023. Institutional ethical approval was given, and the study was conducted in compliance with US Animal Welfare Act guidelines for research. During this timeframe, all cats presenting to the emergency department that were diagnosed with ATE, CHF, or ATE plus CHF were enrolled in the study. After history confirmed no prior administration of antithrombotics, anticoagulants, or nonsteroidal anti-inflammatories and an absence of previously diagnosed comorbidities, informed owner consent was obtained, and the cats were enrolled in the study.
Based on the discretion of the attending veterinarian, these cats may have received sedation (butorphanol tartrate), diuretics (furosemide [Salix]), or analgesia (methadone hydrochloride or fentanyl citrate) prior to blood collection for the study. Any cat who did not tolerate blood collection and exhibited evidence of decompensation was unenrolled from the study, and the collection was aborted for reasons of patient safety. A diagnosis of ATE was confirmed through a blood glucose differential of >30 mg/dL between the thoracic and pelvic limbs to ensure objective diagnostic criteria. Examination findings that increased the index of suspicion of ATE in the attending veterinarian, resulting in a blood glucose differential being performed, included pulselessness, lack of doppler blood flow, lack of bleeding from a cut distal limb, and discoloration of the paw pads and nail beds compared to unaffected limbs. Cardiomegaly was diagnosed via echocardiography with confirmation of LA enlargement (LA:aortic [LA:Ao] ratio > 1.6). An LA:Ao ratio > 1.6 was set as an arbitrary cutoff for the purposes of this study. This was our institutional cutoff based on a previous study showing that an LA diameter of 16.5 mm yielded a specificity and sensitivity of 87% for diagnosing heart failure. Coupled with a previously noted mean aortic root diameter of 11 mm in feline patients, this generates a cutoff LA:Ao ratio for LA enlargement of 1.6. This view was obtained through the right parasternal short axis transaortic view with a 2-D ultrasonography setting, with measurements taken at the end of atrial diastole. Cardiac ultrasonography was performed by either a board-certified veterinary cardiologist or an emergency critical care resident trained by a specialist cardiologist in the acquisition of appropriate views. CHF was diagnosed by thoracic radiography as interpreted by a veterinary radiologist or through the presence of increased lung water (B-lines) or pleural/pericardial effusion on point-of-care thoracic ultrasound in combination with LA enlargement as determined by the 16.5-mm cutoff.

During the study timeframe, cats presenting to the cardiology department who had evidence of LA enlargement without evidence of heart failure and who had not previously received thromboprophylaxis were enrolled into the study. Healthy controls who had not previously received thromboprophylaxis were enrolled into the study. Healthy controls or used for other clinical diagnostic testing. The remaining 0.4 mL was introduced without anticoagulants or activators into a prewarmed cartridge of the VCM unit within 60 seconds of collection. This choice of 60 seconds as a cutoff was employed to ensure standardization of testing. The VCM unit provides results regarding the kinetics, rate, and strength of the developing clot as both a trace and series of values. The results provided for clot time (CT), clot formation time (CFT), a angle, amplitude at 10 minutes after CT (A10), amplitude at 20 minutes after CT (A20), maximum clot firmness (MCF), and lysis index at 30 minutes (L130) and 45 minutes (L145) were recorded, tabulated, and used for statistical analysis for each group.

The VCM unit was located separately from other laboratory equipment that may have caused vibrations (eg, centrifuges) and potentially altered results. Cartridges were warmed on the manufacturer-provided warming pad to 37°C and were discarded if they had spent greater than 4 weeks at this warmed temperature without being used per manufacturer guidelines. Manufacturer recommendations for sample handling, equipment operation, and quality control were followed throughout the study period.

**Statistical analysis**

Statistical analysis was performed using a commercial statistical software package (NCSS, version 2023; NCSS LLC). Statistical testing and analysis were selected and performed by a statistician. Descriptive statistics were generated to characterize the study population. There were 8 variables assessed by the VCM (CT, CFT, a angle, A10, A20, MCF, L130, and L145), and there were 2 factors (the presence or absence of ATE and/or CHF) that were potentially associated with these response variables.

Breed, gender, and age were assessed as possible covariates in the model. If $P < .20$ for breed, age, or gender for a given variable, it was retained in the analysis. The cutoff of $P < .20$ was selected due to the low statistical likelihood of influence on the response variable. Normality of errors was assessed through a normal probability plot. Homogeneity of variance was assessed with a residual*Yhat plot. Data analysis was by means of factorial analysis of variance. If the initial analysis showed a significant difference for a given response variable, post hoc analysis was performed by means of a Tukey test and evaluation of the least square means from the ANOVA.

If non-normal or heterogeneous variance was noted, a Kruskal-Wallis ANOVA was employed for the comparison of groups without covariates. Post hoc analysis of Kruskal-Wallis was by means of a Dunn test. If a significant covariate was noted, a parametric ANOVA was utilized for analysis. $P < .05$ was set for statistical significance.

**Results**

**Study population**

Over the 16 months of the study, a total of 109 cats were enrolled. Three of the cats from the
ATE group and 1 CHF cat were excluded due to premature clot formation during blood collection prior to analysis. Four control cats were excluded due to poor tolerance of sampling or clot formation during blood collection. The 4 cats with HCM (and LA enlargement > 1.65 cm) without evidence of CHF were excluded from data analysis as the small numbers drew concerns for excessive type II errors and poor statistical power to draw conclusions from a group of this size. This left 97 cats included for data analysis: 35 healthy controls (24 male, 11 female), 28 with CHF (19 male, 9 female), 21 with ATE (15 male, 6 female), and 13 with ATE and evidence of CHF (ATE plus CHF) (8 male, 5 female). All cats enrolled were neutered. The most commonly occurring breed was Domestic Shorthair, with 72 cats; the remainder was made up of 7 Domestic Medium hairs, 6 Domestic Longhairs, 4 Maine Coons, 3 Ragdolls, 2 American Shorthairs, 2 Siamese, and 1 Sphynx. The healthy controls were significantly younger than the clinically affected cats (5.6 vs 10.6; P < .01).

Descriptive statistics were generated; normally distributed data had means and SDs generated for data analysis (Table 1), and non-normally distributed data had medians and ranges utilized for further data analysis (Table 2).

Neither breed nor sex were significantly related to any of the response variables (all P values > .20). Therefore, we concluded that breed and sex do not affect any of the variables in this dataset. Age was added as a covariate to account for the differences in age between the test populations. Age was not noted to be significantly associated with CT, CFT, α angle, or MCF but was noted to be significantly associated with A10, A20, Li30, and Li45 (P < .05) and was included as a covariate for analysis of these variables.

### Table 1—Comparison of hemostatic variables pertaining to speed of clot formation, clot strength, and kinetics between cats with aortic thromboembolism (ATE), congestive heart failure (CHF), both diseases, and healthy controls using a benchtop viscoelastic coagulometer (VCM-Vet [VCM]; Entegrion).

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 35)</th>
<th>CHF (n = 28)</th>
<th>ATE (n = 21)</th>
<th>ATE + CHF (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT (s)</td>
<td>285.26 ± 172.64</td>
<td>391.71 ± 106.76</td>
<td>415.9 ± 109.2</td>
<td>368.62 ± 232.64</td>
</tr>
<tr>
<td>CFT (s)</td>
<td>190.77 ± 106.13</td>
<td>190.07 ± 75.67</td>
<td>183.05 ± 52.78</td>
<td>203.69 ± 73.65</td>
</tr>
<tr>
<td>α Angle (degrees)</td>
<td>53.57 ± 9.66</td>
<td>50.14 ± 9.88</td>
<td>51.57 ± 6.92</td>
<td>49.31 ± 9.22</td>
</tr>
<tr>
<td>A10 (VCM units)</td>
<td>27.71 ± 6.73</td>
<td>29.93 ± 9.45</td>
<td>27.67 ± 6.71</td>
<td>27.85 ± 7.43</td>
</tr>
<tr>
<td>A20 (VCM units)</td>
<td>36.74 ± 8.02</td>
<td>37.18 ± 11.82</td>
<td>36.0 ± 7.56</td>
<td>37.31 ± 8.86</td>
</tr>
<tr>
<td>MCF (VCM units)</td>
<td>43.49 ± 9.44</td>
<td>42.79 ± 8.97</td>
<td>39.76 ± 8.07</td>
<td>40.77 ± 9.95</td>
</tr>
</tbody>
</table>

A10 = Amplitude at 10 minutes after CT. A20 = Amplitude at 20 minutes after CT. CFT = Clot formation time. CT = Clot time. MCF = Maximum clot firmness.

### Table 2—Comparison of hemostatic variables pertaining to fibrinolysis between cats with aortic thromboembolism (ATE), congestive heart failure (CHF), both diseases and healthy controls using a viscoelastic coagulation monitor.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 35)</th>
<th>CHF (n = 28)</th>
<th>ATE (n = 21)</th>
<th>ATE + CHF (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li30 (% of MCF)</td>
<td>100 ± 99–100</td>
<td>100 ± 99–100</td>
<td>100 ± 98–100</td>
<td>100 ± 100–100</td>
</tr>
<tr>
<td>Li45 (% of MCF)</td>
<td>100 ± 97–100</td>
<td>99 ± 89–100</td>
<td>98 ± 88–100</td>
<td>98 ± 92–100</td>
</tr>
</tbody>
</table>

Li30 = Lysis index at 30 minutes. Li45 = Lysis index at 45 minutes. MCF = Maximum clot firmness.

### Clot time

The CT dataset was noted to be normally distributed and have good homogeneity of variance. The interaction between CT and both ATE and CHF were noted to be significant (P = .02); as such, post hoc comparisons were employed. Using post hoc analysis, the least square means of the ANOVA were calculated, and a Tukey post hoc test was utilized. The CHF group was noted to have significantly prolonged CT compared to the controls unadjusted (P < .01) and retained significance with a Tukey post hoc analysis (P = .04). The CT for the ATE group was also noted to be significantly prolonged when compared to the control with post hoc analysis (P = .02). There was no significant difference between the ATE plus CHF and control or between any of the heart disease groups (P > .05). A graphical representation of the results is found in Figure 1.

### Clot formation time

There was a reasonable degree of normality and homogeneity to the dataset. There was a single data point that was aberrantly high; however, the dataset was otherwise normal and considered appropriate for analysis. There were no significant differences noted between any of the tested populations for the CFT variable (P > .05). A graphical representation of the results is found in Figure 1.

### α Angle

Age was noted to be a covariate (P = .11) in preliminary analysis. The least square means were adjusted to mean age (8.8 years), and analyses were based on this mean age. It was noted that the α angles of the groups with CHF, with and without ATE, were slightly but not significantly (P = .06) lower than the non-CHF groups, the controls, and the ATE group. A graphical representation of the results is found in Figure 1.
Amplitude at 10 minutes after CT, A20, and MCF

The dataset generated for these 3 variables was noted to have good normality and homogeneity. There were no significant differences between any of the tested populations for these variables ($P > .05$). A graphical representation of the results is found in Figure 1.

Lysis index at 30 minutes

The Li30 dataset was noted to be non-normal in distribution with a heterogeneous variance. Age was noted to be a significant covariate ($P = .01$), so this was included in the ANOVA model. A nonparametric Kruskal-Wallis test was employed to assess the dataset. Although a nonparametric Kruskal-Wallis test is unable to detect a possible interaction as it is a one-way ANOVA, an interaction was viewed to be relatively unlikely due to the high $P$ value ($P = .33$) when assessing the measured variables. There was no evidence of any difference between the different population groups by means of a nonparametric Kruskal-Wallis test. There was also no difference between these groups with a parametric ANOVA to account for age as a covariate. A graphical representation of the results is found in Figure 2.
Lysis index at 45 minutes

Significant differences between ATE, ATE plus CHF, and control groups were noted with a nonparametric Kruskal-Wallis test. A Dunn test was applied for post hoc analysis, and significance was set at $z > 2.63$ due to multiple comparisons. ATE was noted to be significantly hyperfibrinolytic at Li45 compared to control ($z = 3.55$), as was ATE plus CHF ($z = 3.06$). CHF was not noted to be significantly different from controls ($z = 2.56$). When age was applied as a covariate with the parametric ANOVA, this significant difference was no longer present. A graphical representation of the data is found in Figure 2.

Discussion

We hypothesized that cats with ATE and/or CHF would be detectably hypercoagulable when compared to healthy controls using the VCM. The data collected in this study did not support the hypothesis, and there was noted to be some degree of hypocoagulability in the CT variable. Significant Li45 hyperfibrinolysis was detected in the ATE and ATE plus CHF groups, but this was lost when age was applied as a covariate. There were no other significant differences between the populations examined in this study.

Our secondary hypothesis was that cats with HCM without CHF would be detectably hypercoagulable when compared to healthy controls using the VCM. This hypothesis was ultimately not able to be tested due to the small sample size of the subclinical HCM cats presented in the study period. The fact that the VCM did not detect hypercoagulability in the more severely affected cats with cardiac disease (those with ATE and CHF) makes it less likely in the authors’ opinion that there would have been detectable differences in this subclinical population.

This is the first study evaluating hypercoagulability in cats with heart disease and ATE using a viscoelastic testing device. Hypercoagulability was investigated in feline patients with heart disease when compared to healthy controls. In contrast to previously published data showing biomarker evidence of hypercoagulability, there was no evidence of hypercoagulability in this population using the VCM device. The presence of hypercoagulability in feline heart disease patients is suspected to be highly likely given the high incidence of thromboembolic disease in this patient population.

Although it is possible that the thromboembolic complications come purely from the previously demonstrated vascular flow stasis and endothelial injury portions of Virchow’s triad, it seems unlikely that there is a completely normocoagulable state in these animals. This is supported by the previously demonstrated biomarker evidence of hypercoagulability. It is possible that the VCM lacks the necessary sensitivity to correctly identify hypercoagulability in this patient population. The previous scientific abstract presented at a conference utilized TEG and was able to demonstrate statistically significant hypercoagulability in the cats in its cohort. Based on the differences in findings between these studies, it is possible that TEG is more sensitive at detecting hypercoagulability in the ATE patient when compared to VCM. Previous studies have noted that the VCM and TEG testing modalities are poorly correlated; further studies with paired samples run on VCM and TEG would be invaluable to further evaluate this.

Alternatively, there may be other population factors in that study that resulted in their different findings. It must be noted that the average (both median and mean) of each of the patient populations were within manufacturer-published reference ranges for all the tested variables. Indeed, of the 768 datapoints measured, only 25.5% were outside the manufacturer reference range. Whether these specific reference ranges are insensitive at detecting hypercoagulability and relative risk of thrombosis or the testing mechanics of the VCM...
unit are unable to detect hypercoagulability cannot be determined from this study.

The VCM is run on fresh whole blood, and there is no current protocol for the addition of activators or inhibitors. This prevents the flexibility inherent in TEG/rotational thromboelastometry (ROTEM), which would enable direct evaluation of subparts of the tested patients’ coagulation status. This may render the VCM insensitive at detecting hypercoagulability in this patient population. Alternatively, a platelet function analyzer (PFA) or multiple electrode platelet aggregometry, which yield a more direct evaluation of platelet aggregation and hyperactivity, may enable a more direct assessment of the hypothesized platelet-mediated hypercoagulability in feline heart disease patients. The VCM was employed in this study due to its relative ease of use, lower cost, and the logistical institutional access challenges associated with sourcing TEG or ROTEM.

It is possible that employing tissue plasminogen activator–modified TEG or a similar targeted viscoelastic test could yield a more complete evaluation of the differences in fibrinolysis between the investigated populations. Further research with age-matched controls or an evaluation of fibrinolysis throughout various ages in clinically healthy cats would be an effective means to evaluate whether age is a factor in feline fibrinolysis and hyperfibrinolysis or if it is related to the cardiac or thromboembolic dysfunction of these clinical cats.

There were several cats excluded from data analysis due to premature clot formation or an inability to perform atraumatic venipuncture. It is unclear if the premature clot formation was due to a technique issue or if this represents a more severe hypercoagulable state in those patients. As the premature clot formation precluded evaluation of the sample with the VCM unit, significant conclusions regarding the cause of this cannot be drawn.

When evaluating the CT, there was noted to be a significant difference between control and ATE and CHF but not ATE plus CHF. The CTs in these test groups were noted to be prolonged when compared to controls, suggesting a degree of hypocoagulability. The CTs for the ATE plus CHF group were also prolonged but not statistically significantly so. The CT primarily evaluates the clotting factor activity and the enzymatic processes of secondary hemostasis. These have been shown to be elevated in feline cardiomyopathy patients in previous studies. Elevated TAT, D-dimers, and FDPs have all been demonstrated in cardiomyopathy cats. It was somewhat surprising to find that the clinical cases in this study were relatively hypocoagulable when compared to the control group in the CT variable. It is possible that the VCM is insensitive to evaluating this response variable, or the control cats may have been relatively hypercoagulable for an unknown reason (occult heart disease, renal dysfunction, inflammation, or neoplasia). Another possibility is that these cats express a degree of hypocoagulability due to the consumption of their available clotting factors; however, this is considered less likely due to the previously demonstrated biomarker evidence of hypercoagulability in these patients. Paired evaluation of the VCM and TEG, ROTEM, or PFA with other markers of coagulability, such as D-dimers, FDPs, or TAT complexes, would have been an invaluable tool to further characterize this finding and should be utilized in future research.

The α angle was also slightly less in cats with heart failure when compared to those without (both the control and ATE without heart failure groups). This was noted to be statistically insignificant; however, it may have been a type II error due to the relatively small sample size. Hyperfibrinolysis has not previously been associated with this response variable, or the control cats may have been relatively hypercoagulable for an unknown reason (occult heart disease, renal dysfunction, inflammation, or neoplasia). Another possibility is that these cats express a degree of hypocoagulability due to the consumption of their available clotting factors; however, this is considered less likely due to the previously demonstrated biomarker evidence of hypercoagulability in these patients. Paired evaluation of the VCM and TEG, ROTEM, or PFA with other markers of coagulability, such as D-dimers, FDPs, or TAT complexes, would have been an invaluable tool to further characterize this finding and should be utilized in future research.

Interestingly, there was no statistical difference in fibrinolysis between the groups; however, there was potential hyperfibrinolysis detected at the Li45 portion in the cardiac disease groups. Hyperfibrinolysis has not previously been associated with thromboembolic disease in veterinary patients. It is normal for fibrinolysis to upregulate following coagulation activation, such as postsurgically, as the ongoing hemorrhage/vascular injury has ceased, thus allowing blood flow to be re-established following thrombus dissolution. Physiologic fibrinolysis can progress to hyperfibrinolysis when an imbalance between fibrinolytic activators and inhibitors occurs, such as during disseminated intravascular coagulation, sepsis, or vascular occlusion or secondary to trauma. This hyperfibrinolysis can be localized or systemic depending on the severity of the imbalance. Previous human literature has shown that hyperfibrinolysis post-trauma carries higher mortality and higher transfusion requirements. Similar veterinary studies are lacking and have, to date, failed to demonstrate similar evidence of hyperfibrinolysis in cats.

It is important to consider that when age was applied as a covariate to this data analysis, there was no longer a significant difference in the Li45. It is possible, but in the authors’ opinion unlikely, that cats become progressively hyperfibrinolytic as they age. Indeed, the fact that age was significantly associated with A10, A20, L130, and Li45 but not CT, CFT, α angle, and MCF makes it difficult to
draw conclusions from this dataset. It is possible that certain coagulation and fibrinolysis parameters are altered by patient age; however, this cannot be separated from the important pathophysiological abnormalities inherent in the ATE and CHF patient populations. A repeat study with a wider range of ages in the healthy control group, age matching of controls, or research into coagulation and fibrinolysis of healthy cats throughout their lifespan could provide a more definitive conclusion on this matter. It is suspected that the test populations were truly hyperfibrinolytic when compared to the controls. This is supported by previous literature that found cardiomyopathy cats to have elevated concentrations of fibrinolytic biomarkers, such as FDPs, D-dimers, and TAT complexes. Although the cats in this study were not noted to be hypercoagulable on the VCM, this degree of hyperfibrinolysis supports previous studies demonstrating biomarker evidence of hyperfibrinolysis in feline cardiomyopathy patients.

It is possible that the failure to detect systemic hypercoagulability in feline cardiomyopathy cases reflects its absence rather than a true failure of detection. It is possible that local hypercoagulability confined to the left atrium, in combination with the previously described endothelial damage and vascular stasis, facilitates the formation of thrombi, which then displace to cause ATE. The presence of SEC due to erythrocyte aggregation within the left atrium represents localized vascular stasis, and a known risk factor for atrial thrombi and subsequent ATE. The only way to effectively detect this local hypercoagulability would be to perform viscoelastic testing upon samples of blood collected from within the dilated left atrium, which is unlikely to be feasible in these unstable patients.

The study population reflects previous reports of ATE and cardiomyopathy in the published literature, with average ages of 10 to 11 years and with male cats predominating (68% of cardiomyopathy cases). This male bias is likely due to the increased incidence of HCM in males when compared to females. Interestingly, the healthy controls also had a 68% incidence of males, and as such, sex was not noted to significantly relate to any variable in this study. This is potentially a type II error due to a small sample size.

There were insufficient purebred cats in the study cohort to draw statistically significant conclusions regarding breed incidence. Previously, Abyssinian, Birman, Ragdoll, Maine Coon, Himalayan, Siamese, and Persian cats have been shown to be overrepresented in CHF/ATE populations. Other studies have shown the majority of cases to be mixed breed/nonpedigree, although this is suspected to be more related to the relative infrequency of pedigree cats in those study populations rather than true increased relative risk.

It has been previously reported that 69% to 95% of ATE cats have underlying heart disease, and there are, to date, no large-scale studies evaluating other causes. The majority of cats had no history of heart failure/heart disease, with hyperthyroidism and neoplasia being other previously noted predisposing diseases. Given this over-representation of heart disease patients in the ATE population, and the relatively high (11%) frequency of ATE in feline cardiomyopathy patients, it seems a logical conclusion that this disease process is a significant risk for thromboembolic complications. The point at which feline cardiomyopathy patients developed an increased risk of thromboembolism has not been definitively proven, and identification of a hypercoagulable state would be invaluable in further characterizing this disease process. It was our intention to enroll a larger number of asymptomatic HCM patients as this is the population that would benefit most from early identification of hypercoagulability to guide thromboprophylactic therapy. As the VCM was unable to detect hypercoagulability in cats with evidence of thromboembolic disease, it is considered unlikely that these results would have been different in the asymptomatic HCM cats.

There were several limitations to this study. The relatively low numbers of cases reduced the power of our investigation and increased the risk of a type II error in our statistical analyses. The healthy control cats were not from a specific pathogen-free colony, and the possibility of underlying undiagnosed inflammatory or infectious diseases or occult heart disease cannot be excluded. There was no standardized baseline lab work (complete blood count, urinalysis to rule out proteinuria, thyroid testing, biochemical analysis to assess for chronic renal dysfunction) performed in the cats enrolled in this study; this could have potentially included cats with comorbidities associated with a hypercoagulable state in both the controls and clinical cases. The clinically affected cats did not receive a full echocardiogram as part of the study protocol, meaning a definitive diagnosis of HCM versus other cardiomyopathies, which may have differing degrees of coagulation influence, could not be made. There was also no blood pressure testing performed in the cats enrolled, which could have overlooked hypertension-related endothelial damage and thrombotic risk. The current study also did not evaluate biomarkers of coagulation (TAT, D-dimers, vWF) that would have allowed comparison to previous assessments of hypercoagulability in the ATE population.

There was also a significant difference in the ages between the clinical cats and controls, which made drawing conclusions regarding potential L445 hyperfibrinolysis difficult. Younger cats were enrolled as healthy controls to reduce the potential incidence of age-related comorbidities, such as renal dysfunction and heart disease. As this population of cats was exclusively made up of employee pets, the scope for age matching with clinical cases was limited. Repeating this study with age-matched control and clinical groups would hopefully reduce this confounding variable. In the clinical groups, there was no standardization of treatments received prior to blood collection; although any cats who had received medications that are already established to...
interfere with coagulation were excluded, potential alterations due to the authorized medications cannot be definitively ruled out.

Future studies investigating hypercoagulability in feline cardiomyopathy patients should consider utilizing viscoelastic tests more validated in feline patients, such as TEG or ROTEM, or testing modalities with better direct measurement of platelet functionality, such as PFA, platelet agregometry, or multiple electrode platelet agregometry. Further testing at the time of heart failure diagnosis and serial monitoring after the administration of anti-thrombotic therapy (clopidogrel/Xa inhibitors) may enable the assessment of the efficacy of therapy and potentially quantify the relative risk of ATE development. Future studies combining viscoelastic testing with biomarker evidence of hypercoagulability (D-dimers/FDPs/TAT) or evidence of immunothrombosis (NETs/cell-free DNA/citrullinated histones) may increase the sensitivity in identifying hypercoagulability in feline cardiomyopathy patients.

In conclusion, there was no statistically significant viscoelastic evidence of hypercoagulability detected with the VCM in any cats with heart disease when compared to healthy controls. There was some factor-derived hypocoagulability suggested by the CT of some of the clinical cats when compared to controls. There was also mild Li45 hyperfibrinolysis of the clinical cats compared to controls; however, this was no longer clinically significant with age applied as a covariate. Further studies are required to better understand the potential hypercoagulable state in feline cardiomyopathy and aortic thromboembolism patients.

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Disclosures

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