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Original Study

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Evaluation of coagulation status in dogs with naturally occurring canine hyperadrenocorticism

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Abstract

Objective – To determine whether or not there are differences in coagulation parameters (eg, thrombelastography [TEG], activated partial thromboplastin time [aPTT], prothrombin time [PT], and fibrinogen) among dogs with naturally occurring hyperadrenocorticism (HAC), dogs with HAC undergoing medical management, and dogs without HAC.
Design – Prospective, observational study.
Setting – Veterinary teaching hospital.
Animals – Forty-six client-owned dogs undergoing adrenal function testing.
Interventions – None.
Measurements and Main Results – Nine dogs were diagnosed with HAC de novo, 19 dogs were presented for therapeutic monitoring of previously diagnosed HAC, and 18 dogs did not have HAC. Variables compared between groups were age, body weight, platelet count, mean platelet volume, serum concentrations of cholesterol, triglycerides, antithrombin, PT, aPTT, fibrinogen, and TEG parameters (eg, alpha angle, *R*, *K*, and maximum amplitude [MA]). Dogs with HAC and dogs treated for HAC had higher serum cholesterol than dogs without HAC ($P < 0.05$). All groups had mean MA greater than the institutional reference interval. There was a weak, positive correlation between hematocrit and MA that was independent of diagnosis ($r^2 = 0.266$, $P = 0.004$).
Conclusions – The results of this study do not support the supposition that a significant difference exists in coagulation tendencies between dogs with HAC prior to treatment, dogs with HAC during treatment, and dogs without HAC. This disagreement with the classically accepted notion that HAC leads to a hypercoagulable state could be due to a couple of possibilities. Namely, the link between HAC and hypercoagulability may be relatively weak, or our findings may be the result of a type II error either as a result of a small sample size or the use of coagulation assays that are insensitive to the effects of HAC on the hemostatic system.

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Introduction

Naturally-occurring canine hyperadrenocorticism (HAC), or canine Cushing's syndrome, is a relatively common endocrinopathy in dogs characterized by chronic hypercortisolemia.[1] The recognized causes for HAC in the dog include ACTH-secreting pituitary tumors (ie, pituitary-dependent HAC) or cortisol-secreting tumors arising from the adrenal cortex (ie, functional adrenal tumor). Hypercortisolemia can also result from chronic exogenous glucocorticoid administration (ie, iatrogenic HAC).

HAC leads to a constellation of common clinical signs including polyuria/polydipsia, polyphagia, a "potbellied" appearance, and alopecia, and may also manifest with less-frequent clinical presentations such as

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calcinosis cutis and myotonia.[1] Additionally, the systemic effects of excessive glucocorticoid concentrations may predispose patients to complications such as diabetes mellitus, recurrent infections (eg, of the urinary tract or skin), poor wound healing, and weakening of connective tissues, which may lead to disorders such as cranial cruciate ligament rupture.[2] The presence of these systemic manifestations of disease justifies a firm recommendation to begin medical therapy. The presence of other clinical signs that negatively affect the owner or patient's quality of life (eg, polyuria and polydipsia) also justifies therapeutic intervention. However, a subset of patients with HAC may have few clinical signs, or experience minimal impact on quality of life from their disease.[2] This may be most evident in dogs that are diagnosed in the process of investigating incidental findings on routine blood work, or are diagnosed in the process of ruling out more progressive and potentially serious medical diseases. In these patients, the veterinarian and owner face the more difficult decision of whether the cost and inherent risk of therapy is justified by the (presumed minimal) response to treatment.

In the human as well as veterinary literature, it is generally accepted that HAC leads to a hypercoagulable state.[1] Pulmonary thromboembolism (PTE) in particular is suggested to be a complication of naturally occurring HAC, but there are conflicting reports as to the frequency and etiology of thromboembolic disease in these patients.[3–9] Clinically, it is difficult to demonstrate hypercoagulability, which in turn makes it challenging to study this tendency in a large population of affected animals. The uncertainty surrounding this risk factor makes it difficult to make recommendations regarding the necessity of thromboprophylaxis in patients with clinical signs of HAC. Strong proof of hypercoagulability in mildly affected animals may also provide better justification for institution of therapy for HAC in this subset of patients.

There is poor documentation of the actual incidence of PTE in combination with HAC in the veterinary literature. The epidemiologic evidence that HAC leads to an increased incidence of PTE and thromboembolic disease in dogs is primarily based on anecdotes, case series,[3,8,9] and retrospective studies of dogs positively diagnosed with PTE.[4,5,7] In many of these reports, the risk for developing thromboembolic complications in dogs was inferred from the described association of thromboembolic disease in people with HAC.

Thrombelastography (TEG) is a viscoelastic technique for the assessment of global coagulation function that has been investigated in many veterinary species, using a variety of methodological techniques.[10–13] By monitoring the tension created by the fibrin strands of a developing thrombus, TEG allows investigators to evaluate the rate of clot formation as well as the strength of the final clot using whole blood. Whole blood models are superior to plasma-based tests of coagulation because they include the contributions of cellular components of the blood such as platelets and microparticles. TEG is one of the few techniques that can identify patients who have an increased tendency toward clot formation (ie, hypercoagulability), in addition to identifying patients who are hypocoagulable or who have normal coagulation function.[14] TEG tracings that are deemed hypercoagulable may display any or all of the following characteristics: a shortened reaction time (ie, time to formation of initial fibrin strands), an increased rate of clot formation, or an increased final clot strength, while the opposite is true of hypocoagulable tracings.[14] Although TEG does not describe the specific etiology of hypercoagulability in different clinical states, it does allow the investigator to identify a tendency toward hypercoagulability in individuals and specific populations. Because different aspects of the TEG tracing represent contributions of different aspects of the hemostatic system, changes in clinical patients may be attributed more to the cellular (eg, platelet) or the soluble (eg, coagulation factor) aspects of hemostasis.

The purpose of this study was to assess and compare coagulation function in 3 groups of dogs: dogs with naturally occurring HAC prior to medical or surgical intervention, dogs with naturally occurring HAC monitored during medical therapy for HAC, and dogs screened for HAC that were found not to have the disease. Because results generated by TEG do not quantify an objective risk for thrombosis, it was considered more realistic to compare dogs with HAC to other clinic patients as opposed to comparing them to preestablished reference intervals. Our hypothesis was that there are no statistically significant differences in coagulation parameters (eg, TEG, activated partial thromboplastin time [aPTT], prothrombin time [PT], fibrinogen concentration) among dogs with naturally occurring HAC, dogs with HAC undergoing medical management, and dogs from a hospital population in whom the diagnosis of HAC has been ruled out.

Materials and Methods

Study population

Dogs were considered for inclusion if there was a clinical indication to assess adrenal function via baseline cortisol concentration, ACTH stimulation test, low-dose dexamethasone suppression test (LDDST), or an extended adrenal panel.[a,15,16] These tests were performed to evaluate dogs suspected of having hypoadrenocorticism, suspected of having HAC, or undergoing therapeutic monitoring for previously diagnosed HAC. Because subjects

were clinical patients presenting to the UGA-VTH under a variety of clinical scenarios, it was not possible for all samples to be collected under fasting conditions. Informed consent was obtained prior to enrollment of patients, and the University of Georgia clinical research committee approved all aspects of this study.

Exclusion criteria included failure to obtain informed consent, body weight <2 kg, anesthesia or surgery within 10 days prior to evaluation, tumor invasion of the vena cava confirmed by diagnostic imaging, surgical exploration, or necropsy and atypical HAC (defined by a normal serum cortisol concentration with concurrent increases of other adrenal analytes), and nondiagnostic ACTH stimulation results (ie, poststimulation serum cortisol concentration between 18 and 22 μg/dL [497–607 nmol/L]). Ongoing therapy with glucocorticoids was not a defined exclusion criterion because such patients would not typically have been considered for adrenal function testing.

Provocative adrenal function testing was performed as described,[2] and cortisol measurements were performed using an immunoassay.[b] LDDST was performed using 0.01 mg/kg of dexamethasone[c] IV, and ACTH stimulation testing was conducted using synthetic ACTH[d] at a dose of 125 μg IV for patients weighing <20 kg and 250 μg IV for patients weighing >20 kg. A positive diagnosis of HAC (HAC dogs) was made based on compatible clinical signs and history, consistent abdominal imaging when performed, and positive results of provocative adrenal function testing: post-ACTH stimulation serum cortisol concentration >22 μg/dL (607 nmol/L) or 8-hour post-LDDST serum cortisol concentration >1.4 μg/dL (39 nmol/L).

Dogs that had met these diagnostic criteria for HAC prior to the existence of our study, and that presented for evaluation of ongoing HAC medical therapy for during the time of our study were included in the HAC-tx group. Dogs with test results that excluded the diagnosis of HAC (dogs without HAC) were compared with dogs in the HAC and HAC-tx categories.

Sample collection

Blood was collected for analysis at the first instance of sampling if multiple samples were collected during the patient's hospitalization. Blood was collected via atraumatic direct jugular venipuncture, from peripheral vessels using a butterfly catheter, or from an indwelling central venous catheter. When samples were obtained from indwelling catheters, they were either obtained from catheters that had not yet been exposed to heparinized saline, or a discard sample of 3 times the volume of the catheter was removed prior to acquisition of the diagnostic sample.[17] Approximately 6 mL of whole blood was collected and divided between two 1.8 mL tubes containing 3.2% sodium citrate[e] (final ratio of blood to citrate = 9:1) and a tube without additives.[e] One tube with citrated blood was allowed to rest at room temperature for 30 minutes prior to analysis by TEG. The remaining blood samples were centrifuged for 10 minutes at 4°C (39°F) at 1,500 × *g*. The supernatants (ie, citrated plasma or serum) were immediately separated into polypropylene tubes and stored at −80°C (−112°F). After TEG analysis was initiated, the remaining citrated whole blood was analyzed for hematocrit, platelet count, and mean platelet volume (MPV)[f] (if not performed already as part of the clinical evaluation), with a multiplier of 1.1 for all counts to adjust for the additional dilution of the citrate.[18] Serum was assayed for cholesterol and triglyceride concentrations,[g] and citrated plasma was assayed for antithrombin activity,[g] PT,[h] aPTT,[h] and fibrinogen[h] concentration. All plasma or serum assays were conducted within 8 months of initial collection (samples were stored following collection and submitted for batch analysis). Additional patient data collected for statistical analysis included age, body weight, and serum cortisol concentrations obtained with provocative adrenal function testing.

Thrombelastography

Samples were run in duplicate using 1 of 2 dedicated TEG machines[i] at a time. For each analysis, 340 μL of citrated whole blood was added to 20 μL of 0.2 M $CaCl_2$[j] in warmed (37°C, 99°F) TEG cups without heparinase.[k] All tracings were recorded for a minimum of 120 minutes, or until the maximum amplitude (MA) had been reached. The variables reported for this study are reaction time (*R*, min), clot formation time (*K*, min), angle (α, degrees), and maximum amplitude (MA, millimeters).[14]

Statistical analysis

Data were analyzed using a commercial software program.[l] Data were assessed for normality using the Kolmogorov-Smirnov test. Normally distributed data were analyzed using 1-way ANOVA. Data that were not normally distributed were analyzed using Kruskal-Wallis ANOVA on ranks, and significant relationships were further evaluated using Pairwise Multiple Comparisons Procedures (Dunn's method). Categorical values were analyzed using a chi-square test. Significance was set at $P < 0.05$.

Results

A total of 51 dogs were evaluated for inclusion in the study; 46 dogs satisfied the inclusion criteria. One dog was excluded because it had surgery less than 10 days

prior to evaluation, 1 dog had an adrenal mass identified to be invading the caudal vena cava, and 3 dogs had post-ACTH stimulation serum cortisol concentrations between 18 and 22 μg/dL (497–607 nmol/L).

Nine dogs were diagnosed with HAC, 19 dogs presented for monitoring of treatment for previously diagnosed HAC (HAC-tx), and 18 dogs did not have HAC (non-HAC). Of the non-HAC dogs, 4 had undergone cortisol measurements because they were suspected of having hypoadrenocorticism (2 were diagnosed as having hypoadrenocorticism), and 14 had undergone cortisol measurements because they were suspected of having HAC.

Variables compared between groups were age, body weight, platelet count, MPV, serum cholesterol, serum triglycerides, antithrombin, PT, aPTT, fibrinogen, and TEG parameters.

TEG variables for each group are listed in Table 1. Although there were no statistical differences between groups, the mean MA for each group was greater than the institutional reference interval. The remaining TEG parameters were within institutional reference intervals. There was a statistically significant, indirect relationship identified between MA and HCT, when comparing values for all dogs ($r^2 = 0.266$, $P = 0.004$; Figure 1).

There were no statistically significant differences between groups in measured coagulation parameters, including platelet count ($P = 0.74$), and mean values were within the institutional reference interval for all groups (Table 2). The only statistically significant difference between groups was serum cholesterol; the median cholesterol concentration was 5.34 mmol/L (206 mg/dL) (range 4.9–6.4 mmol/L [191–248 mg/dL]), reference interval 3.3–6.8 mmol/L (129–264 mg/dL) in the non-HAC compared with 9.74 mmol/L (376 mg/dL) (range 6.3–42.2 mmol/L [244–1,628 mg/dL] in the HAC group, $P < 0.05$), and when comparing the non-HAC group with the HAC-tx group (median 7.38 mmol/L [285 mg/dL], range 3.3–13.7 mmol/L (126–530 mg/dL), $P < 0.05$).

As shown in Table 3, 89% (8/9) of HAC dogs had an MA greater than the reference interval, compared to 68% (13/19) of HAC-tx and 78% (14/18) of non-HAC dogs. This difference was not statistically significant ($P = 0.483$). Likewise, 44% (4/9) of HAC dogs displayed a *K* value below the reference interval, compared to 21% (4/19) of HAC-tx dogs and 17% (3/18) of non-HAC dogs. This difference was also not statistically significant ($P = 0.261$). For alpha angle, 22% (2/9) of HAC dogs had a value above the reference interval, compared to 11% (2/19) of HAC-tx dogs, and 17% (3/18) of non-HAC dogs. This difference was not significant ($P = 0.96$). No dog in any group had an *R* time outside of the reference interval.

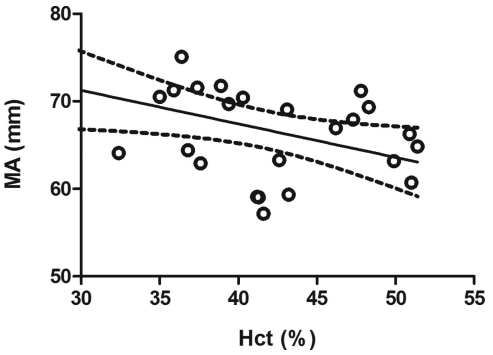

Figure 1: Correlation between maximum amplitude (MA) and hematocrit (HCT) in dogs evaluated for hyperadrenocorticism. Ninety-five percent confidence band for the line of best fit; a mild positive correlation was detected ($r^2 = 0.266$, $P = 0.004$) that is independent of diagnosis.

Discussion

The results of this study do not support the premise that there is a difference in coagulation tendencies between dogs with HAC prior to treatment, dogs with HAC during treatment, and dogs without HAC presenting to a referral clinic for evaluation. Of note, many of the dogs studied were hypercoagulable, based on comparison with our institutional reference interval for TEG. As this reference interval was derived using young (ie, dogs between 2 and 5 y of age) healthy adult dogs, it suggests either the need for an age-specific reference

Table 1: Thrombelastography results in dog evaluated for adrenal dysfunction. Normally distributed data (eg, MA) are presented as mean ± SD; data not normally distributed are presented as median (range). Reference intervals in parentheses are for normal dogs in our laboratory.

	HAC	Non-HAC	HAC-tx
R (min)(2.1–11.0)	5.0 (2.45–12.1)	6.3 (2.7–13.9)	5.05 (2.65–14.0)
K (min)(1.2–4.6)	1.35 (0.85–4.1)	1.73 (0.8–4.1)	1.7 (0.95–5.0)
Angle (degrees)(39–74)	70.15 (39.8–76.6)	66.63 (42.7–78.6)	65.95 (35.8–75.7)
MA (mm)(44.5–61.7)	67.26 ± 4.9	66.13 ± 6.0	65.24 ± 5.6

HAC, dogs with hyperadrenocorticism; HAC-tx, dogs undergoing treatment for previously diagnosed hyperadrenocorticism; non-HAC, dogs without hyperadrenocorticism.

Table 2: Selected biochemical, hematology, and coagulation function testing results in dogs evaluated for adrenal dysfunction. Normally distributed data are presented as mean ± SD; data not normally distributed are presented as median (range). Reference intervals (RIs) in parentheses are for normal dogs in our laboratory.

	HAC	Non-HAC	HAC-tx
Cholesterol (mmol/L)(RI: 3.34–6.8 mmol/L)(RI: 129–264 mg/dL)	9.74 (6.32–42.17)(376 [244–1628])*	5.34 (4.95–6.42)(206 [191–248])	7.38 (3.26–13.73)(285 [126–530])*
Platelet ($\times 10^9$/L)(RI: 235–694 $\times$ 10^9/L)(RI: 235–694 $\times$ $10^3/\mu$L)	497 ± 173(497 ± 173)	447 ± 196(447 ± 196)	433 ± 142(433 ± 142)
Antithrombin (%)(108–146)	133 ± 19.7	126 ± 24.1	134 ± 9.7
PT (sec)(5.8–9.8)	7.4 ± 0.6	7.5 ± 0.5	7.2 ± 0.5
aPTT (sec)(9.4–15.1)	11.3 ±1.4	12.1 ± 1.4	11.0 ±1.0
Fibrinogen (μmol/L)(RI: 4.4–11.7 μmol/L)(RI: 150–400 mg/dL)	11.2 ± 2.7(381 ± 90)	10.38 ± 3.0(353 ± 103)	8.8 ± 2.5(300 ± 86)

*Indicates statistically significant difference from non-HAC group ($P < 0.05$).
HAC, dogs with hyperadrenocorticism; HAC-tx, dogs undergoing treatment for previously diagnosed hyperadrenocorticism; non-HAC, dogs without hyperadrenocorticism.

Table 3: Abnormalities in TEG parameters within patient groups. Data are shown as number (percent) of dogs with a value outside the reference range for that parameter.

	Mean MA > 61.7 mm	Mean *K* < 1.2 minutes	Mean angle > 74 degrees	Mean *R* < 2.1 minutes
HAC ($n = 9$)	8 (0.89)	4 (0.44)	2 (0.22)	0
Hac-tx ($n = 19$)	13 (0.68)	4 (0.21)	2 (0.11)	0
Non-HAC ($n = 18$)	14 (0.78)	3 (0.17)	3 (0.17)	0

HAC, dogs with hyperadrenocorticism; HAC-tx, dogs undergoing treatment for previously diagnosed hyperadrenocorticism; non-HAC, dogs without hyperadrenocorticism.

interval for TEG values, or, more intriguingly, the idea that dogs presenting to a secondary or tertiary referral hospital with signs consistent with systemic illness may be relatively hypercoagulable, independent of adrenocortical functional status.

Serum cholesterol was the only statistically different variable between groups, with non-HAC dogs having statistically lower serum cholesterol compared with dogs with HAC and dogs with HAC undergoing treatment. While patients were not universally fasted prior to blood draw, and postprandial hyperlipidemia (as opposed to persistent, fasting hyperlipidemia) may confound this finding, it is also true that HAC dogs are typically hypercholesterolemic.[1] In people, atherosclerosis (an inflammatory vascular disease) is associated with an imbalance in circulating lipids, particularly an increase in low-density lipoproteins.[19] The metabolic changes associated with HAC might lead to vascular pathology, which could in turn activate the coagulation cascade.[m]

We demonstrated a significant correlation between the HCT and the TEG MA value. This has previously been reported in both the human and veterinary literature, and is thought to result from a relative increase in plasma protein compared to red blood cell numbers, allowing the formation of a stronger, faster clot as HCT decreases.[20,n,o,p] Although this is a concern when interpreting TEG data from variably anemic patients, in this study, all patients had HCT within a relatively narrow reference interval, and the ranges were not significantly different between groups, allowing comparison in our study.

In the veterinary literature, the increased risk of thromboembolic disease attributed to HAC is based primarily on 2 retrospective studies focused on PTE.[4,5] In one study,[5] 8 of 47 dogs with a necropsy diagnosis of PTE and a complete medical record were diagnosed with HAC. However, only 3 of them had a diagnosis of HAC without comorbid diseases.[5] Multiple disease processes were identified in 64% of dogs.[5] In the second retrospective study, Johnson et al[4] reported that 2 of 57 dogs with necropsy-confirmed PTE had a clinical diagnosis of HAC, which represented 2.7% of all dogs with HAC necropsied during the study period. Because the focus of that study was pathologic findings associated with PTE (as opposed to thrombosis in HAC), it is unclear whether the dogs with HAC had complete medical records or concurrent diseases. It is reported that multiple diseases were present in 59% of patients.[4] Because of the retrospective nature of these studies, and the selection bias inherent in electing to perform necropsies, it would be inappropriate to assume causality between HAC and PTE based on these reports alone.

Support for the anecdotal claim that HAC in dogs is associated with increased risk of thrombosis may be

sought in the human medical literature. However, in the human medical field, the majority of studies and case reports document thrombosis and thromboembolic disease as complications of surgical management of HAC, as compared to the spontaneous PTE anecdotally described in HAC dogs.[21,22] While a single case report[23] documents recurrent thromboembolism in a patient diagnosed with HAC that resolved with successful medical and surgical treatment of the underlying disease, this patient had multiple comorbid risk factors for the development of thrombosis (eg, hypertension, obesity, surgery). Casonato et al[24] also assessed the response to transsphenoidal hypophysectomy, and reported a worsening of hypercoagulability for 3 months postoperatively before normalization, which may corroborate some anecdotal reports in veterinary medicine of thrombosis occurring after the initiation of medical therapy for HAC.[24] Conversely, a study comparing postoperative complications in human patients with HAC to an age-matched control group failed to document an increase in risk of thromboembolism.[25]

Effectively documenting a predisposition toward thrombosis in a patient population can be a challenging endeavor. McCrath et al[26] determined that MA as measured using TEG was a strong predictor of postoperative thrombotic complications (specifically myocardial infarction) in people when applying a cutoff MA value of 68 mm.[26] Diagnosing thrombosis of a single large vessel is not always a diagnostic challenge, but the diagnosis of systemic thrombosis (arterial or venous) relies heavily on tests that lack sensitivity and specificity such as plasma D-dimer concentrations, or the use of imaging techniques such as contrast angiography and nuclear scintigraphy that are not commonly employed in clinical veterinary practice. More frequently, thrombosis is a clinical diagnosis, or one made at necropsy. The possibility that other tests, such as thrombin-antithrombin complexes[27] or the quantification of platelet or endothelial microparticles, may be more specific for the occurrence of thrombosis or endothelial damage merits further investigation toward a reliable test for thrombotic predisposition in veterinary patients.

Multiple studies in dogs attempting to attribute hypercoagulability to increased cortisol concentrations have investigated the hemostatic system. Authors have proposed varying theories on the mechanism for a cortisol-induced hypercoagulability, including imbalance between procoagulant and anticoagulant factors, as well as decreased fibrinolysis.[27–29] The procoagulant factors that veterinary medical authors have investigated related to this procoagulant state include factors II, V, VII, IX, X, XII, and fibrinogen.[27,29] In human HAC patients, the tendency toward hypercoagulability has been attributed to increases in factor activity for factor VIII alone,[22,30] the combination of factors VIII, V, and prothrombin,[21] factors XII, XI, IX, and VIII[31] and increased factor VIII and von Willebrand factor (vWF) activity with abnormal vWF multimers and hyperresponsive platelet activity.[24] Decreased function of the anticoagulant system has been attributed to a decrease in antithrombin concentrations.[27] Additionally, authors have attributed the hypercoagulable state seen in people with HAC to a decreased fibrinolytic potential due to increased plasminogen activator inhibitor (PAI-1).[28,32] Many of the studies evaluating the canine hemostatic system were performed in dogs without definitively diagnosed thrombosis, making it difficult to positively correlate the abnormalities described with the development of thromboembolic disease. The correlation between HAC and a hypercoagulable state in the dog has not previously been rigorously documented, although our clinical impression is that, if such a correlation does exist, it is an infrequent and inconsistent finding.

Proving the presence or absence of a hypercoagulable state ex vivo poses certain challenges. Coagulation is a dynamic process that relies on the interaction between soluble factors in the circulation, red blood cells, platelets, the endothelium and endothelial microparticles, and vascular tone. While in vivo and in vitro tests are readily available that can rapidly diagnose a hypocoagulable state (eg, buccal mucosal bleeding time [BMBT], activated clotting time [ACT], PT, aPTT), similarly reliable tests able to document a hypercoagulable state do not exist. A correlation has not been established between abnormally low (rapid) results of clotting function tests and a predisposition to thrombosis. While TEG shows promise as a global evaluation of whole blood coagulation status, it remains an ex vivo test, and one that is unable to assay the effects of endothelial damage or shear stress on the initiation of a clot. Thus, it seems possible that failure to document hypercoagulability by ex vivo TEG measurement does not completely exclude an in vivo phenomenon, such as endothelial activation, that could be promoting coagulation in HAC patients. The converse is also true; systemic factors within the patient could attenuate the effects of any disturbances measured by TEG. However, in a study of HAC dogs with no clinical signs indicating activation of the clotting cascade, Jacoby et al[27] were able to document a significant increase in thrombin-antithrombin complexes, compared with non-HAC control dogs. Thrombin-antithrombin complexes indicate thrombin formation and were, therefore, interpreted as markers of thrombosis, which was otherwise subclinical.[27] One trend that is apparent in the medical and veterinary literature is the disparity in significant findings between studies; while many authors pursue the hypothesis that HAC predisposes to thrombosis, it is clear that a consistent shift in the balance of

the hemostatic system has not been repeatably defined in either people or dogs with HAC.

Thrombocytosis is a common hematologic abnormality associated with canine HAC,[33] and thrombocytosis has been associated with an increased risk for thrombosis in dogs.[34] An increase in platelet count has also been shown to increase clot strength as measured by TEG.[35] It follows that if dogs with HAC are truly predisposed to thrombosis, it may be in part due to the tendency toward thrombocytosis. Further investigation would require separate evaluations of platelet count and function in dogs with naturally occurring HAC. Interestingly, platelet counts between groups in this study were not statistically different.

Systemic hypertension is considered a common finding in dogs with HAC,[36,37] which could lead to an increase in shear stress of the small arteries and arterioles and exposure of the subendothelial collagen. In turn, this could activate the coagulation cascade and lead to thrombus formation. Systemic blood pressure was not measured as part of this study and future studies should be designed to evaluate systemic blood pressure as a possible comorbid condition contributing to an increased tendency to form clots.

There are several limitations of the present study. The preliminary sample size calculation based on a theoretical prevalence of hypercoagulability of 0.5 indicated that 12 patients per study group would provide adequate power to detect a clinically relevant difference of 10 mm in MA. Based on hospital records at the time of the study design, a new diagnosis of canine HAC was made in approximately 30 patients per year. One year was budgeted for patient enrollment, but unfortunately, the case volume at our institution declined during the course of the study, and only 9 patients were enrolled into the HAC group during the study period. While it is possible that enrollment of 12 HAC patients may have affected the results, it seems more appropriate to recommend that future studies of this phenomenon should use a lower estimate for prevalence of hypercoagulability in their sample size calculations.

As previously discussed, patients were not universally fasted prior to blood collection. Additionally, systemic arterial blood pressure was not included as part of the study design, which may have been an important comorbid factor. Sample collection was performed by direct venipuncture and the use of central lines, both of which can introduce variability into measures of coagulation.

The most significant limiting factor, however, is that currently there is no universally accepted testing methodology for performing TEG in veterinary medicine. Particularly, the use and type of a coagulation trigger (ie, activator) is a subject of much debate among investigators. Our clinical protocol that was employed in this study uses recalcification to allow coagulation to proceed, rather than using a strong activator such as tissue factor. One concern with our methodology is that it may preferentially reflect the behavior of ex vivo factors, over in vivo hemostatic capability. Without a strong activator, and relying on simple contact of the recalcified sample with the cup and pin, coagulation may have been variably initiated among the samples due to differences in intrinsic pathway activation. While a more robust coagulation activator may have enhanced the detection of certain hypercoagulable tendencies, the anecdotal claims of hypercoagulability in dogs with HAC have not been attributed to any specific factors in vivo. The downside of using high concentrations of a strong activator such as TF is that subtle changes in the activity of many of the factors that may be responsible for the alleged hypercoagulability (factors VIII, IX, XII) may be masked by the generation of thrombin by the extrinsic pathway, effectively negating the need for activation of those factors. It was our intent to confirm or refute the presence of hypercoagulability, due to unknown causes, as a common feature of canine HAC. We, therefore, felt that the more subtle recalcification trigger, and a prospective comparison of affected to unaffected dogs, was the most appropriate means to search for a common phenomenon. However, because the possibility remains that ex vivo factors may have influenced the results of the TEG analyses in this study, these data should be interpreted cautiously.

In conclusion, an association between hypercoagulability and canine HAC, as determined by a whole blood viscoelastic assay, was not documented in this study. There are 3 possible explanations for this finding. First, despite prior anecdotal reports, it is possible that there is no true association. Second, it is possible that recalcification-triggered TEG was insufficiently sensitive to detect differences among groups. It is possible that a hypercoagulable state due to endothelial or other in vivo changes cannot be detected by this modality, and it is conversely possible that ex vivo factors influenced our results. Until convincing evidence documents the superiority of a single methodology for whole blood viscoelastic coagulation testing, all such studies, including ours, must be cautiously interpreted. Finally, it is possible that hypercoagulability only occurs in a subset of HAC dogs, as a function of comorbid factors, such as hypertension, hypercholesterolemia, or diabetes mellitus. Future studies should consider the use of additional assays for coagulation status and endothelial activation, as well as assessment of comorbid factors that may help identify subsets of HAC dogs that are at risk of hypercoagulability and its associated adverse events. Additionally, prospective, controlled longitudinal studies may provide further information on the proposed existence of

an increased relative risk of thromboembolic complications described in canine HAC.

Footnotes

[a] University of Tennessee Clinical Endocrinology Service, Knoxville, TN 37996-4543.
[b] Immulite, Siemens Healthcare Diagnostics, Flanders, NJ.
[c] Dexamethasone, Bimeda, Le Seuer, MN.
[d] Cortrosyn, Amphastar, Rancho Cucamonga, CA.
[e] Citrate and sterile tubes, Becton Dickenson, Franklin Lakes, NJ.
[f] Advia, Bayer Corp, Shawnee Mission, KS.
[g] Hitachi 912: Roche Diagnostics GmbH, Germany.
[h] Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, NY.
[i] TEG 5000, Haemoscope, Niles, IL.
[j] $CaCl_2$, 0.2 M, Haemoscope.
[k] Plain cups and pins, Haemoscope.
[l] Sigma Stat 3.5, Systat software, Inc, Chicago, IL.
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