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Characterization of thrombelastography over time in dogs with hyperadrenocorticism

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ABSTRACT

Canine hyperadrenocorticism (HAC) is a common endocrinopathy often associated with hypercoagulability, thrombosis and thromboembolism and it can contribute to increased morbidity and mortality. The condition results in increased, unregulated secretion of glucocorticoids (GCs). While prospective identification of hypercoagulability is challenging, thrombelastography (TEG) is a diagnostic tool that enables the detection of hypercoagulability in a clinical setting. The objective of this prospective cohort study was to serially assess coagulation in dogs with HAC using TEG to test the hypothesis that dogs with HAC have increased TEG maximal amplitude (MA) and that the MA would normalize once clinical control was achieved. Twenty-three dogs with naturally occurring HAC were enrolled and hemostatic (including TEG, platelet function, thrombin–antithrombin complexes and coagulation panel) and hematological variables were measured at presentation. TEG was serially monitored until clinical resolution of HAC was attained.

At presentation, most dogs with HAC had increased MA values, increased thrombin–antithrombin complexes and many were hyperfibrinogenemic. Platelet function analyzer-100 (PFA-100) closure times were significantly prolonged. TEG tracings did not normalize in either medically- or surgically-managed dogs, but fibrinogen concentrations decreased. It seems that dogs with HAC have a complex coagulopathy in which hypercoagulability and platelet hyporeactivity or dysfunction might occur simultaneously. As TEG tracings did not normalize in well-controlled dogs, it is unlikely that increased blood GCs are solely responsible for TEG alterations seen in dogs with HAC.

Introduction

Hyperadrenocorticism (HAC) is a common endocrinopathy in dogs that results in increased, unregulated secretion of glucocorticosteroids (GC). In dogs, the most common cause of HAC is an adrenocorticotropic hormone (ACTH) secreting pituitary adenoma, which results in bilateral adrenal hyperplasia. GC-secreting adrenal tumors are recognized less commonly (Reusch, 2005).

A potentially fatal complication of HAC in dogs is thrombosis and thromboembolism of both the arterial and venous systems including aortic (Van Winkle et al., 1993; Boswood et al., 2000), pulmonary (Burns et al., 1981; LaRue and Murtaugh, 1990), caval (Teshima et al., 2008), portal (Respess et al., 2012) and splenic (Hardie et al., 1995) vascular beds. Studies investigating the pathophysiological pathways of altered hemostasis in dogs and people with HAC have demonstrated systematic increases in procoagulant factor activities and fibrinogen concentrations (Feldman et al., 1986; Jacoby et al., 2001; Caldin et al., 2009; Van Zaane et al., 2009; Klose et al., 2011). Hypercoagulability puts dogs at risk for thrombosis; however, it is challenging to make an accurate clinical and laboratory diagnosis of a hypercoagulable state.

Thrombelastography (TEG) is an in vitro diagnostic technology that integrates the cellular and soluble components of the hemostatic process to yield a global assessment of the hemostatic potential. The technology is based on the ability to sense and record changes in the viscoelastic properties of blood while it clots. To do this, a torsion wire is suspended in a rotating cup that contains a whole blood specimen. A number of comprehensive reviews of TEG in veterinary medicine have been published recently (for example, Kol and Borjesson, 2010).

TEG has been validated for use in several veterinary species, including the dog (Wiinberg et al., 2005; Bauer et al., 2009). It is used increasingly in veterinary medicine to identify and proactively manage animals that are predisposed to thrombosis. Increased maximal amplitude (MA) values have been documented in healthy dogs receiving GCs (Rose et al., 2011) and in dogs with various inflammatory, degenerative and neoplastic disorders

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(Otto et al., 2000; Wiinberg et al., 2008; Wagg et al., 2009; Goodwin et al., 2011; Vilar Saavedra et al., 2011; Goggs et al., 2012). An increased MA has also been reported in dogs with newly diagnosed HAC, in dogs that had been actively treated for HAC, and in a control group of sick dogs with unspecified diseases (Klose et al., 2011).

Platelet function in dogs with HAC has not been extensively studied. A single small study reported that dogs with HAC had increased platelet aggregation in response to adenosine diphosphate (ADP) stimulation (Halmay et al., 2008). A recent study suggests that while GCs do not affect platelet aggregation, ACTH might augment it directly (Pozzi et al., 2009). Overall, the data are conflicting, with reports suggesting that GCs either do not alter platelet function (Dal Bo Zanon et al., 1983), or that GC administration in vivo or in vitro results in platelet hyporeactivity (Ikkala et al., 1985) or hyperreactivity (Glass et al., 1981).

The objective of this study was to document TEG tracings in dogs with HAC from initial presentation until clinical resolution. We hypothesized that dogs with newly diagnosed HAC would have increased MA values and that TEG tracings would normalize with clinical remission.

Materials and methods

Study design

This prospective cohort study was approved by the Clinical Trial Review Board of the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California, Davis (UCD, VMTH). All dog owners were informed and signed a consent form. Blood was initially sampled at the time of HAC diagnosis, a second sample was taken during induction therapy (for those treated medically), and a final blood sample was drawn once clinical resolution had been achieved.

Animals

Dogs were enrolled between January 2009 and February 2010. All dogs were client-owned, were presented to the UCD VMTH, and had naturally occurring disease. The diagnosis of HAC was based on history, physical examination, and the results of a complete blood count (CBC; ADVIA-120 hematology analyzer, Siemens Medical Solutions Diagnostics), biochemical profile (Hitachi 917 chemistry analyzer, Roche), complete urinalysis performed on urine collected by antepubic cystocentesis (Urisys 1800, Roche), low dose dexamethasone suppression test (LDDST), urine cortisol:creatinine ratio, and abdominal ultrasound. Diagnostic threshold values of serum cortisol concentration post LDDST > 40 nmol/L and of urine cortisol:creatinine ratio >32 were used to establish the diagnosis of HAC. The diagnosis of pituitary-dependent hyperadrenocorticism (PDH) or adrenal-dependent hyperadrenocorticism (ADH) was based on the presence of bilateral adrenal enlargement or a unilateral adrenal mass, respectively, as assessed by abdominal ultrasonographic scan. Serum cortisol concentrations were measured using an Immulite 2000 (Siemens Medical Solutions Diagnostics).

None of the dogs had been treated with non-steroidal anti-inflammatory drugs or adrenolytic drugs at the time of presentation. Relevant clinical data (i.e. clinical evidence of hemorrhage, thrombosis, coagulation disorders and clinical resolution status) were recorded. For dogs managed medically, clinical resolution was defined as improvement or resolution of clinical signs of HAC and a post-ACTH stimulation serum cortisol concentration $\leqslant$200 nmol/L, or $\leqslant$140 nmol/L for dogs treated with trilostane or mitotane, respectively. For dogs undergoing adrenalectomy for a cortisol-secreting adrenocortical tumor, clinical resolution was defined as resolution of clinical signs following discontinuation of oral prednisone treatment. The study design is illustrated in Fig. 1.

Sample collection

On initial presentation, minimally traumatic blood samples (6 mL) were collected via jugular or cephalic venepuncture, using a 20 G needle and a 6 mL syringe, from dogs for which there was a strong clinical suspicion of HAC prior to LDDST. Blood was drawn prior to LDDST to avoid potential effects that dexamethasone might have on TEG. Blood was then placed in an EDTA tube, a serum collection tube with no additive (Tyco Healthcare Group) and two 3.2% sodium citrate tubes (BD vacutainer).

TEG and PFA-100 analyses

TEG analysis was performed at the UCD VMTH using a TEG 5000 (Haemoscope Corporation) and data were analyzed using software version 4.2 according to the manufacturer's specifications. Internal quality control procedures, including control specimens provided by the manufacturer, were run with every test specimen. Specimens were placed at room temperature for 30 min prior to analysis and kaolin was used as an activator, as previously described (Bauer et al., 2009). For more background TEG information, see Kol and Borjesson (2010) and Wiinberg and Kristensen (2010). TEG results were compared to a canine reference interval previously developed at the UCD VMTH (n = 25 dogs, assayed in duplicate). The reference population was comparable to test population in regards to age, sex and breed.

Platelet function testing was performed using the Platelet Function Analyzer-100 (PFA-100, Dade Behring) using citrated whole blood, as previously validated for use in dogs (Clancey et al., 2009a). Briefly, the PFA-100 measures the time it takes to form a platelet plug in whole blood after exposure to an agonist under high shear stress. This time is reported as closure time (CT; Favaloro, 2008). Collagen and ADP cartridges were used as previously described (Smart et al., 2009). No dog had a platelet count <100 $\times$ 10^9/L. Results from dogs with HAC were compared to results obtained from 14 healthy, age-matched control dogs, based on history, clinical examination and normal CBC results. When CTs were infinite, samples were re-run for confirmation.

Plasma based coagulation tests

Citrated whole blood was centrifuged (2500 g, 10 min, RT7 plus, Sorval) and plasma was removed, aliquoted and frozen at −80 °C. Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, antithrombin (AT) activity, D-dimer concentration (UCD, VMTH Clinical chemistry laboratory) and thrombin–antithrombin (TAT) complexes (UC Davis Medical Center, Hematology and coagulation laboratory) were determined using standard methods. PT, aPTT, fibrinogen concentration and AT activity were measured by an automated hemostatic analyzer (STA compact, Diagnostica Stago), by coagulometric (PT, aPTT and fibrinogen concentration), or chromogenic (AT activity) methods. D-Dimer concentration was measured via a semi-quantitative, latex agglutination slide immunoassay (Pacific Hemostasis D-Dimer assay, Therom scientific). TAT complexes were measured using an ELISA kit (Enzygnost TAT, Siemens Healthcare Diagnostics) previously validated for use in dogs (Maruyama et al., 2005).

Statistics

The Shapiro–Wilk test was used to determine if data were normally distributed and a nonparametric repeated measures Friedman test was used with a Dunn post hoc test to compare the TEG results and fibrinogen concentrations at the three time points. Spearman correlation was used to determine the correlation between TEG variables and selected hematologic and other hemostatic variables. An unpaired Student's t test was used to compare PFA-100 CT values between dogs with HAC and control dogs. A paired Student's t test was used to compare fibrinogen concentrations at presentation and at clinical resolution. For all tests, P-values <0.05 were considered statistically significant.

Results

Animals

Twenty-three dogs with naturally occurring HAC were enrolled in the study. Seventy percent (16/23) had PDH and 30% (7/23) had ADH. Other than three Labrador retrievers, the remaining dogs included a single dog of each of 13 different pure breed dogs and seven mixed breed dogs. There were 12 spayed females, two intact females, eight castrated males and one intact male dog. The median age at presentation was 10 years (range 3–15 years).

Selected hemostatic and hematologic variables are summarized in Table 1. All dogs (n = 23) had TEG analysis performed on the day of initial presentation. Additional assays were prioritized based on plasma availability and clinical need (Table 1). Ninety-six percent (22/23) of the dogs had increased MA values at presentation. Two of these 22 dogs had mixed TEG patterns consisting of increased MA with a second TEG alteration, indicating hypocoagulability (one dog had increased R (reaction time) and the other had increased R, K (speed of clot formation) and decreased α). Fifty-two percent (12/23) of the dogs had two or more altered TEG variables, consistent with hypercoagulability. Of the 20 dogs with increased MA and no mixed patterns, over half (52%, 11/20)