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ORIGINAL STUDY

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Multicenter investigation of hemostatic dysfunction in 15 dogs with acute pancreatitis

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Abstract

Objective: To characterize hemostatic profiles in dogs with acute pancreatitis.

Design: Prospective and observational study.

Setting: Tertiary referral centers.

Animals: Fifteen client-owned dogs with acute pancreatitis enrolled between December 1, 2011 and June 1, 2012.

Materials and methods: Blood samples were collected on admission for measurement of platelet count, PCV, thromboelastography (TEG), antithrombin, prothrombin time, activated partial thromboplastin time, D-dimer, von Willebrand factor, and fibrinogen values, which were compared to reference intervals derived from healthy dogs. The Wilcoxon rank-sum test was used to test for differences in continuous variables between study subjects and reference intervals.

Measurements and main results: Dogs with acute pancreatitis were globally hypercoagulable using TEG when compared with reference intervals. Dogs with acute pancreatitis had significantly higher D-dimers (1,144 μg/L vs 251 μg/L [6264.5 vs 1374.5 nmol/L]; $P = 0.001$), fibrinogen (837 vs 232 mg/dL [8.37 vs 2.32 g/L]; $P < 0.001$), and von Willebrand factor (92.9% vs 65.1%; $P = 0.02$) as well as significantly lower antithrombin (85.7% vs 120%; $P < 0.001$) and prothrombin time values (3.8 vs 7.6 sec; $P < 0.001$) than reference intervals.

Conclusions: Laboratory evidence of hypercoagulability was present in dogs with acute pancreatitis. TEG may be useful in dogs with acute pancreatitis for monitoring response to therapy and guiding therapeutic interventions.

KEYWORDS

hypercoagulability, D-dimers, fibrinogen, pancreas, canine

1 | INTRODUCTION

Acute pancreatitis is a relatively common disease affecting dogs.[1] The severity of disease from pancreatitis ranges from mild local signs to severe systemic signs and even death. Acute pancreatitis is known to result in systemic activation of coagulation and inflammation.[2–6] Systemic inflammatory response syndrome is associated with the development of microthrombi,[7] which may contribute to subsequent organ failure. Dogs with acute pancreatitis are considered to be at increased risk for thrombotic events, though limited evidence-based research is available on this topic. In one necropsy study of dogs with fatal acute pancreatitis, thrombotic complications were documented

Abbreviations: aPTT, activated partial thromboplastin time; AT, antithrombin; cPL, canine pancreatic lipase; MODS, multiple organ dysfunction syndrome; PT, prothrombin time; TEG, thromboelastography; vWF, von Willebrand factor.

in 38.5% of dogs, and 74% of these dogs had thrombi in multiple organs.[8]

In people with acute pancreatitis, an increase in D-dimers correlates with disease severity, presumably as a marker of intravascular fibrin deposition and thrombi formation.[9] Additionally, platelet count, prothrombin time (PT), fibrinogen degradation products, protein C activity, C-reactive protein concentration, and antithrombin activity (AT) have all been evaluated in varying studies as indicators of disease severity in addition to supporting the pro-inflammatory and hypercoagulable nature of acute pancreatitis in people.[10,11]

In dogs, coagulation changes that accompany acute pancreatitis have not yet been well-defined. While it is suspected that dogs with acute pancreatitis are hypercoagulable, hypercoagulable conditions have until recently been difficult to detect in a clinical setting.[5] Thromboelastography (TEG) has emerged as a useful tool to document the presence and severity of both hyper and hypocoagulable states. The purpose of this prospective study was to evaluate TEG, D-dimer concentrations, coagulation profiles, fibrinogen concentration, vWF, and AT activity in dogs with acute pancreatitis with the hypothesis that affected dogs will have laboratory evidence of hypercoagulability.

2 | MATERIALS AND METHODS

2.1 | Enrollment

Client-owned dogs presenting to 1 of 2 specialty hospitals (Angell Memorial Animal Hospital, Boston, MA or the Foster Hospital for Small Animals at the Cummings School of Veterinary Medicine at Tufts University) between December 1, 2011 and June 1, 2012 with a diagnosis of pancreatitis were eligible for enrollment. A diagnosis of acute pancreatitis was made based on clinical signs (eg, vomiting, abdominal pain experienced within the previous 48 h) in conjunction with at least 1 of the following: (1) abdominal ultrasonographic findings consistent with acute pancreatitis performed by a diagnostic imaging specialist including pancreatitic enlargement, hypoechoic pancreatic tissue suggestive of edema, and hyperechoic mesentery around the pancreas suggestive of inflammation of peripancreatic tissue, or (2) a positive (> 400μg/L) canine pancreatic lipase (cPL).* Additional inclusion criteria included the presence of adequate diagnostic testing to establish severity of disease for the disease severity scores used to evaluate patients, and these values had to be obtained within the first 24 hours of diagnosis (eg, biochemistry profile, urinalysis, complete blood count, lactate concentration, abdominal ultrasound, and blood pressure measurement). Any additional diagnostic tests performed and supportive care decisions were at the attending clinician's discretion. The study was approved by the Institutional Animal Care and Use Committees at each site and owners provided informed consent.

Dogs receiving any medications that may have altered coagulation parameters before the time of initial blood sampling were excluded from the study. These medications included corticosteroids, platelet inhibitors, low molecular weight heparins, heparin, synthetic colloids, or blood products. Dogs with comorbidities known to alter hemostasis (eg, protein losing nephropathy, protein losing enteropathy, immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, Cushing's disease or neoplasia) were also excluded.

2.2 | Blood collection

Following enrollment, whole blood was collected using atraumatic venipuncture and placed into two 2.7 mL citrated tubes to the appropriate 1:9 dilution. Tubes were gently inverted to ensure mixing of citrate with blood. Following a 90 minute rest period (either in the Clinical Science Laboratory or partially in the private vehicle during transport), kaolin-activated TEG was performed.† The 90 minute rest period was chosen to permit time for transfer of the sample by private vehicle from the enrolling institution to the TEG laboratory. The additional tube was centrifuged for 10 minutes at 1620 × *g*, and the citrated plasma was stored at −80°C until batch analysis for aPTT, PT, AT activity, fibrinogen concentration based on Clauss method, vWF, and D-dimer was performed using the Elite coagulation analyzer.‡ PCV was determined locally as part of initial patient assessment, using a microhematocrit tube.

2.3 | Coagulation diagnostics

Kaolin-activated TEG was performed as previously described.[12,13] One milliliter of citrated whole blood was added to a tube containing kaolin activator§ and mixed in accordance with the manufacturer's directions. Next, 340 μL of the kaolin-activated sample was added to the TEG cup containing 20 μL of $CaCl_2$ for a total volume of 360 μL. Analyses were run at 37°C until the MA was reached. Variables recorded were maximum amplitude (MA), reaction time (R), amplification time (K), α-angle (α), and G-value (G) using American Society of Veterinary Clinical Pathology guidelines.[14]

2.4 | Disease severity scoring

Within 24 hours of admission in to the study a preliminary clinical index severity score for dogs with acute pancreatitis,[15] an $APPLE_{fast}$ score,[16] and an organ dysfunction score[17] were completed. Necropsies were performed by a board certified pathologist on all non-survivors to look for evidence of thromboembolic disease or other causes of death.

2.5 | Statistical analysis

The mean values of all dogs were compared to established reference intervals for TEG parameters (R, K, α, MA, and G). The reference intervals used were those established by the Clinical Sciences Laboratory using 25 healthy dogs. Normality was tested for and showed that most variables were not normally distributed, therefore all variables were treated as non-normally distributed throughout the study. Spearman rank correlation coefficients were determined for TEG measurements versus PCV and platelet count. The Wilcoxon rank-sum test was used to compare TEG between reference values and study dogs. Statistical significance was set at $P < 0.05$ for all tests. All analyses were performed using commercial statistical software¶ used for all analyses.

TABLE 1 Thromboelastographic variables of 15 dogs with naturally occurring acute pancreatitis in comparison with reference intervals

Variables	Reference intervals	Study dogs	*P*-value
R (min)	4.5 (2.0)	3.8 (0.8)	0.008
K (min)	2.1 (0.7)	1.2 (0.7)	<0.0001
α (deg)	62.0 (10.0)	73.2 (10.7)	<0.0001
MA (mm)	54.6 (7.0)	68.3 (10.9)	0.0002
G (d/sec)	6,006 (1,643)	10,800 (6,100)	0.0002

Values are presented as median (interquartile range).

3 | RESULTS

Fifteen dogs were included in the study: 8 neutered females, 6 neutered males, and 1 intact female. The median age was 9 years (range 3–14 years). Of these dogs, 13 had ultrasonographic findings consistent with pancreatitis. Eight dogs had consistent ultrasonographic and cPL testing combined. Two dogs were enrolled based on a positive cPL test with appropriate clinical signs. Thirteen dogs survived to discharge and 2 dogs died. One dog died from complications associated with its pancreatitis, and the other was euthanized due to failure to respond to treatment and a perceived poor prognosis.

The median length of hospital stay was 5 days (range 2–13 days). For the 2 dogs that died, the median initial MODS score was 3, the median initial $Apple_{fast}$ score was 5, and the median initial Canine Pancreatitis Score was 8. For the 13 dogs that survived to discharge, the median initial MODS score was 1, the median initial $Apple_{fast}$ score was 1, and the median initial Canine Pancreatitis Score was 5. One non-surviving dog developed an arterial thromboembolism (ATE) during hospitalization.

All dogs were hypercoagulable on TEG analysis. TEG variables in affected dogs are listed in Table 1. Dogs with acute pancreatitis had a higher MA ($P = 0.0002$), shorter R time ($P = 0.008$), shorter K time ($P < 0.0001$), greater α ($P < 0.0001$), and higher G values ($P = 0.0002$) when compared with reference intervals.

Comparison of coagulation indices in the affected dogs with reference intervals is shown in Table 2. Dogs with acute pancreatitis had significantly higher D-dimers ($P < 0.001$), fibrinogen concentrations ($P < 0.001$), and vWF antigen activity levels ($P = 0.02$) as well as significantly lower AT activity ($P < 0.001$) and PT ($P < 0.001$) than reference intervals There was no difference in aPTT between dogs with acute pancreatitis and reference intervals ($P = 0.74$). This study did not find any association between platelet counts or PCV and any of the individual TEG parameters.

4 | DISCUSSION

The results of the present study documented coagulation abnormalities in dogs with acute pancreatitis, with changes in whole blood kaolin-activated TEG supporting a hypercoagulable state. TEG has been used to describe the coagulation changes seen in disseminated intravascular coagulation,[18] parvovirus,[19] immune-mediated hemolytic anemia,[20,21] immune-mediated thrombocytopenia,[22] protein-losing enteropathy,[23] septic peritonitis,[24] and protein-losing nephropathy.[25]

TEG characterized dogs with acute pancreatitis as hypercoagulable, with a significantly higher MA, α-angle, and G values and significantly shorter R and K times compared to reference intervals. While clinically acute pancreatitis has been associated with thromboembolic disease and suspected hypercoagulabilty,[7] this study provides confirmation of hypercoagulablity in a whole blood assay from patients with confirmed pancreatitis.

Conventional plasma-based coagulation tests including PT and aPTT are most helpful for the detection of hypocoagulability.[25,26] While one study found an association between shortened PT and aPTT and some indicators of hypercoagulabilty,[27] there has yet to be proof that shortened PT and aPTT are adequate for predicting hypercoagulability. In the present study, dogs with acute pancreatitis had significantly shortened PT compared to reference intervals but did not have significant changes in aPTT. One possible contributing factor to this could be preactivation of samples. Another possible explanation could be that shortened PT is indeed an indicator of hypercoagulability and this theory should be further explored future studies.

Dogs with acute pancreatitis also had significant differences in coagulation tests other than TEG. The D-dimer concentration was significantly higher in dogs with acute pancreatitis in comparison with reference intervals. Increased D-dimers are consistent with breakdown of fibrin, indicating that there has been an increase in the formation of clots.[28] In people, with acute pancreatitis increases in D-dimer concentration is a consistent prognostic indicator, with

TABLE 2 Median hemostatic values of 15 dogs with naturally occurring acute pancreatitis in comparison with reference values

Variables	Reference intervals	Study dogs	*P*–value
Platelet count $x10^3/\mu L$ [$x10^9/L$]	150–450	310 (2,234)	0.44
PCV (%)	37–50	46.5 (40.0)	0.23
PT (sec)*	7.6; 6.1–9.7	3.8 (3.9)	<0.001
aPTT (sec)	14.8; 9.7–19.8	15.4 (77.6)	0.74
Fibrinogen mg/dL* [g/L]	232.0; 117.0–455.0 [2.32; 1.17–4.55]	837.0 (1,040) [8.37 (10.40]	<0.001
D-dimer μg/mL* [nmol/L]	2.51; 1.21–5.47 [13.74; 6.63–30.0]	11.44 (44.72) [62.65 (244.88)]	<0.001
vWF antigen (%)*	65.1; 33.2–109.0	92.9 (124.10)	0.02
Antithrombin activity (%)*	120.0; 89.0–146.0	85.7 (66.7)	<0.001

Values are presented as median (interquartile range).

higher concentrations being significantly more common among non-survivors.[29]

The median fibrinogen concentration was significantly higher in dogs with acute pancreatitis. Interestingly, hypofibrinogenemia is expected in people with disseminated intravascular coagulation, but in people with acute pancreatitis a rise in D-dimers correlates with an accompanying rise in fibrinogen.[9] Fibrinogen is a precursor to the formation of fibrin, and the 2 main causes of hyperfibrinogenemia include hemoconcentration and inflammation.[30] During inflammatory disease processes the liver produces fibrinogen as an acute phase protein. The authors are not able to make any conclusions on mechanisms for the rise in fibrinogen in the dogs in this study, but assume its rise is correlated with the hypercoagulable initial phase of the inflammatory state in these patients as no other causes of specific hyperfibrinogenemia were found.

Median AT activities less than 71% are assosciated with a significantly higher mortality and rates of MODS in people with acute pancreatitis.[29] The antithrombin protein normally serves as an anticoagulant in the body, blocking propagation of clot formation in the cell-based model of coagulation.[31] The AT activity of dogs with acute pancreatitis was lower than reference intervals in this study. This was expected and is consistent with the pro-inflammatory and hypercoagulable state seen in the dogs with acute pancreatitis in this study.

vWF antigen activity is used as a prognosticator and indicator of generalized endothelial damage in inflammatory conditions in people.[32,33] High vWF antigen activity may be associated with low concentrations of ADAMTS13, but ultimately the higher vWF antigen activity contributes to a hypercoagulable state.[34] It has also been found to be increased compared with normal in dogs with sepsis.[35] In this study, dogs with acute pancreatitis had significantly higher vWF antigen activity when compared with reference intervals, suggesting that endothelial damage might be responsible for some changes observed.

Limitations in this study include the small sample size, thereby limiting of the reliability of the statistical analyses, using reference intervals rather than having a control population of healthy dogs at the same time of the study, specific case presenting complaints and diagnostic results (instead using a checklist format), that only a single sample was collected, variability in duration and severity of disease which was not recorded, and the low number of non-survivors, which limited the ability to identify positive prognostic results. However, this study confirms the previous belief that dogs with acute pancreatitis have an in vivo hypercoagulable condition, and this knowledge may impact the way clinicians manage the condition in the future. Additional studies are recommended to assess a larger population of dogs with acute pancreatitis for changes in these values over time. This may be useful for monitoring response to therapy in addition to predicting mortality and development of MODS. The potential role for mitigating thrombotic risk with anticoagulants from acute pancreatitis in dogs remains unclear, but further investigation is advised.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ENDNOTES

* SNAP cPL TEST, IDEXX Laboratory, Westbrook, ME.

† TEG 5000 Hemostasis Analyzer, Haemonetics, Braintree, MA.

‡ Elite Coagulation Analyzer, Instrumentation Laboratories, Bedford, MA.

§ Kaolin, Haemoscope Corp, Niles, IL.

¶ SAS 9.2, SAS Institute, Cary, NC.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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