Original Study

Assessment of the relationships among coagulopathy, hyperfibrinolysis, plasma lactate, and protein C in dogs with spontaneous hemoperitoneum

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Abstract

Objective – To relate coagulation and fibrinolysis derangements to shock severity as reflected by plasma lactate concentrations in dogs with spontaneous hemoperitoneum (SHP) and determine the impact on transfusions.
Design – Prospective, observational, case-control study.
Setting – Three veterinary teaching hospitals.
Animals – Twenty-eight client-owned dogs with SHP and 28 breed- and age-matched control dogs.
Interventions – None.
Measurements and Main Results – Blood samples for platelet counts, coagulation, and anticoagulant assays (prothrombin time, activated partial thromboplastin time, fibrinogen, antithrombin, and protein C, thromboelastography [TEG]), fibrinolysis testing (d-dimer and TEG lysis parameters with and without the addition of 50 U/mL of tissue plasminogen activator [TEG LY30 measured with the addition of 50 U/mL of tPA to the blood sample, $LY30_{50}$ and TEG LY60 measured with the addition of 50 U/mL of tPA to the blood sample, $LY60_{50}$; LY30 and LY60]), and plasma lactate as an indicator of severity of shock were collected from SHP dogs at the time of diagnosis. SHP dogs were hypocoagulable (prolonged prothrombin time and activated partial thromboplastin time, decreased TEG maximum amplitude) and hyperfibrinolytic (increased $LY30_{50}$ and TEG $LY60_{50}$) compared to controls. The severity of hypocoagulability was related to protein C activity, while the severity of hyperfibrinolysis was related to plasma lactate concentration. Among the 18 dogs discharged from the hospital, $LY30_{50}$ was significantly associated with the dose of fresh frozen plasma administered, but none of the parameters were associated with the dose of red blood cells administered.
Conclusions – Dogs with SHP have evidence of hypocoagulability, protein C deficiency, and hyperfibrinolysis. Parameters of hyperfibrinolysis were related to plasma lactate concentrations and volume of plasma transfused during hospitalization. These derangements resemble those found in people with acute coagulopathy of trauma and shock, and activation of protein C may be a common feature to both syndromes.

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Keywords: clinical epidemiology, clinical trials, clinical pathology, coagulopathies, coagulation, critical care, fluid therapy, hemostasis, shock, transfusion medicine

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Abbreviations

AAA	abdominal aortic aneurysm
ACoTS	acute coagulopathy of trauma and shock
aPC	activated protein C
aPTT	activated partial thromboplastin time
AT	antithrombin activity
Fg	fibrinogen
$LY30_{50}$	TEG LY30 measured with the addition of 50 U/mL of tPA to the blood sample

LY60$_{50}$	TEG LY60 measured with the addition of 50 U/mL of tPA to the blood sample
PAI-1	plasminogen activator inhibitor-1
PCV	packed cell volume
PT	prothrombin time
SHP	spontaneous hemoperitoneum
TEG	thromboelastogram
TF	tissue factor
tPA	tissue plasminogen activator

Introduction

Up to 30% of human trauma patients develop a severe early coagulopathy, referred to as the acute coagulopathy of trauma and shock (ACoTS), which is associated with a 5-fold increase in mortality.[1,2] People with tissue injury due to trauma accompanied by hypoperfusion develop clotting disorders that have recently been related to activation of the protein C anticoagulant pathway and an associated increase in fibrinolysis, while those without hypoperfusion rarely develop these disorders.[3,4] Trauma patients with hyperfibrinolysis have higher mortality rates (77%) than those without (33%).[5] The CRASH-2 trial suggested that trauma patients treated with an antifibrinolytic drug (tranexamic acid) had lower mortality rates than those who received a placebo, further supporting the relevance of hyperfibrinolysis, although the use of antifibrinolytic drugs for trauma patients remains controversial.[6-8] Hyperfibrinolysis has also been documented in human patients with ruptured abdominal aortic aneurysms (AAA), which results in a syndrome similar to spontaneous hemoperitoneum (SHP) in dogs. In these patients, the severity of hyperfibrinolysis is increased in patients with shock, as assessed by periods of arterial hypotension.[9,10] Although the efficacy has not been well studied, antifibrinolytic agents are commonly used to treat people with AAA to reduce blood loss during surgical repair.[11,12]

SHP is a common emergent syndrome in dogs that requires surgical intervention for definitive therapy.[13] Most commonly, a splenic mass is the source of bleeding, and most dogs receive at least 1 blood transfusion, and many dogs require multiple units of blood and plasma for stabilization.[13] Blood transfusions increase the cost of care, are a potentially limited resource in veterinary practice, and escalating administration of blood products has been identified as an independent predictor of mortality in critically ill people, and is also negatively associated with outcome in dogs with SHP.[13,14] Therefore, therapies to reduce blood transfusion requirements have the potential to reduce cost, morbidity, and mortality in dogs with SHP. The clinical presentation of canine SHP

may be similar to acute hemorrhagic syndromes seen in human patients as they both involve disruption of blood flow and regional tissue ischemia with concurrent systemic hypoperfusion due to hemorrhagic shock. Because of this similarity, we hypothesized that dogs with SHP are at risk for the combination of hyperfibrinolysis and coagulopathy identified in people. Moreover, because dogs are relatively hyperfibrinolytic compared to people because of increased plasminogen activator and decreased plasminogen activator inhibitor-1 (PAI-1) activities, they may be more susceptible to this fibrinolytic pathway imbalance.[15,16]

Diagnosis of hyperfibrinolysis is challenging. While tests for individual fibrinolytic pathway proteins (eg, plasminogen, alpha-2 antiplasmin, tissue plasminogen activator [tPA], PAI-1) may be available through research or referral laboratories, their utility as predictors of in vivo hyperfibrinolysis is limited.[17] Rotational thromboelastometry (ROTEM) has been used to identify hyperfibrinolytic human trauma patients by comparing the degree of fibrinolysis detected when blood samples are tested with and without the addition of a fibrinolysis inhibitor (APTEM vs. EXTEM).[18] Recently, tissue factor (TF) activated thromboelastography (TEG) assays modified with the addition of recombinant tPA have been developed and shown to accurately reflect the fibrinolytic potential of whole blood samples from human patients.[19] This approach has also recently been used to demonstrate possible hypofibrinolysis in dogs with diseases predisposing them to thrombosis.[20] These new assays offer the ability to model fibrinolysis in vitro and apply the techniques clinically at the point of care.

We undertook a study of coagulation and fibrinolysis in SHP dogs with the goals of identifying the hemostatic abnormalities previously described in human trauma patients and providing a rationale for designing therapeutic treatment trials to improve patient outcomes. Assessment of dogs was undertaken using both traditional coagulation testing and a modified TEG assay for evaluation of whole blood fibrinolytic potential. We hypothesized that dogs with SHP would demonstrate hypocoagulability and hyperfibrinolysis compared to age- and breed-matched controls, and that the severity of these disturbances would be associated with the severity of shock, with activation and depletion of protein C, and with increasing transfusion requirements.

Materials and Methods

Animals

Dogs presenting to the emergency services of the 3 participating hospitals with a diagnosis of SHP were eligible for enrollment in the study. Exclusion criteria included known or suspected traumatic hemoperitoneum, severe

thrombocytopenia consistent with immune-mediated thrombocytopenia, NSAID, steroid, or aspirin administration, or presence of diseases known to affect coagulation or fibrinolysis (eg, Cushing's disease, protein losing enteropathy/nephropathy, immune-mediated disease, or previously diagnosed neoplasia not related to the SHP). Hemoabdomen was confirmed by abdominocentesis with retrieval of an effusion with a packed cell volume (PCV) greater than 20%, consistent with standard emergency room protocol. The study was approved by the various institutional animal care and use committees or clinical sciences review committees, and all owners provided informed consent. Blood was sampled at the time of intravenous catheter placement or by peripheral venipuncture. When possible, blood was collected at the time of catheter placement, before the institution of initial fluid therapy. It was not possible to collect blood through the peripheral IV catheter for 3 dogs. For these dogs, samples were drawn by peripheral venipuncture using a 21 Ga butterfly catheter. All blood was drawn directly into vacutainer tubes, starting with an initial red top tube, followed by the citrate tubes. Three 2.7 mL vacutainer tubes containing 0.3 mL of 3.2% buffered sodium citrate were collected for coagulation and fibrinolysis assessment. A heparinized blood sample was collected for venous blood gas and venous plasma lactate measurement (measured within 5 min of venipuncture). Lactate was measured using a blood gas analyzer[a] or handheld point of care device.[b] These 2 devices have previously been demonstrated to have a very high degree of agreement across a clinically relevant range of plasma lactate concentrations in dogs.[21] Finally, an EDTA sample was obtained for measurement of PCV, total plasma protein, and platelet count. All patient management decisions were left to the discretion of the attending clinician and were not affected in any way by enrollment in the study.

One healthy control dog of the same or similar breed and age (within 1 y) was recruited for each dog with SHP. Blood samples were collected via peripheral venipuncture into three 2.7 mL vacutainer tubes containing 0.3 mL of 3.2% buffered sodium citrate for coagulation and fibrinolysis testing, and into a microtainer EDTA tube for PCV and platelet count.

Coagulation and fibrinolysis testing
Three 400 μL aliquots of citrated whole blood were placed in polypropylene tubes and left at room temperature for 30 minutes to be used in thromboelastography analysis. The remainder of the citrated blood was immediately centrifuged at room temperature at 2,500 × g for 20 minutes and the platelet-poor plasma supernatant was aspirated and stored frozen at −80°C up to 6 months before batch analysis at a reference laboratory.[c] Samples were shipped to the reference laboratory overnight on

dry ice and stored at −80°C until thawing at 37°C just before analysis. Coagulation, anticoagulant, and fibrinolysis testing included prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin activity (AT), protein C activity, clottable (Clauss) fibrinogen (Fg), and d-dimer concentration. The matched healthy TEG control dogs were screened for factor deficiencies or dysfibrinogenemias based on PT, aPTT, and Fg determinations. The assays were performed using an automated coagulation instrument[d] and commercial reagents.[e] The results of AT and protein C were reported as the percentage of a pooled normal canine reference plasma (prepared at the Coagulation Lab from 20 healthy dogs) that had an assigned value of 100% activity. Fibrinogen was reported as milligrams per deciliter as measured from a canine plasma standard whose Fg content was determined by gravimetric assay of thrombin-clottable Fg.[22] The d-dimer concentration was measured in reference to a human d-dimer calibration standard.[f]

TF-activated TEG was performed using a thrombelastograph[g] with standard disposable cups and pins. TF[h] was reconstituted with sterile water per manufacturer recommendations, and then diluted using HEPES saline + 2% bovine serum albumin to a final dilution of 1:3,600 in the TEG reaction mixture.[23] To enhance fibrinolysis, human recombinant tPA[i] was added to 2 of the citrated blood aliquots. tPA was reconstituted with sterile water to a concentration of 1 mg/mL (580,000 U/mL) and frozen in 100 μL aliquots at −80°C for a maximum of 6 months until use. A previous study documented that human recombinant tPA reconstituted to 1 mg/mL is stable at −80°C for at least 7 years.[24] The tPA aliquot was thawed at room temperature and further diluted with sterile water. Two dilutions were created such that when 10 μL of the tPA dilution were added to 2 of the 400 μL aliquots of citrated blood, final concentrations of tPA in the TEG reaction mixture of 50 U/mL and 100 U/mL were achieved. To normalize the potential for the tPA to dilute the blood sample, 10 μL of HEPES saline + 2% bovine serum albumin were added to the third 400 μL aliquot (the native sample). For TEG analysis, 20 μL of 10% calcium chloride and 10 μL of diluted TF were pipetted into the warmed (37°C) TEG cups. Thirty minutes after blood collection, 10 μL of either HEPES saline + 2% bovine serum albumin or diluted tPA were added to each 400 μL aliquot of citrated blood, the polypropylene tube was gently inverted 5 times, and 330 μL of the solution was pipetted into the TEG cup. All assays were run until 60 minutes after the clot reached maximum amplitude (MA) and the following TEG parameters were recorded: R time, K time, angle, MA, LY30, and LY60. For the samples to which tPA was added, the parameters were noted with the tPA concentration as a

subscript (eg, TEG R time measured with the addition of 50 U/mL of tPA to the blood sample, R_{100}). The clinicians managing the cases did not have access to the coagulation or fibrinolysis testing, although point of care coagulation testing was available at all institutions and was used at the discretion of the attending clinician.

Statistical analysis

All statistical analyses were done using a commercial statistical software package.[j] Data were tested for normality using the Kolmogorov–Smirnov test. Normally distributed data are reported as mean ± standard deviation, and nonnormally distributed data are presented as median and range. Comparisons of coagulation parameters (PT, aPTT, Fg, R, K, α, MA) AT, protein C, and fibrinolysis parameters (d-dimers, LY30, LY60) between groups were done using the independent samples *t*-test if data were normally distributed. The Mann–Whitney *U*-test was used if data were not normally distributed. Proportions of SHP dogs exhibiting coagulopathy or dysfibrinogenemias were compared to those of control dogs using Fisher's exact test. Linear regression analysis was used to assess the relationship between fibrinolysis and coagulation parameters and the degree of shock as assessed by lactate as well as protein C activities. Stepwise multiple linear regression analysis was used to assess the relative value of lactate (as a measure of severity of shock) and protein C compared to the traditional coagulation testing parameters (PT, aPTT, Fg, d-dimers, AT) and PCV for predicting the degree of fibrinolysis as assessed by the LY30 and LY60. Finally, stepwise multiple linear regression analysis was used to assess the relative ability of admission LY30, PT, aPTT, Fg, d-dimers, AT, and admission PCV to predict the amount of fresh frozen plasma and red blood cells administered to patients sent to surgery. A *P* value of 0.05 was considered statistically significant.

Results

Animals

Twenty-eight dogs with SHP were recruited from the emergency services of the 3 participating institutions between September 2012 and April 2013. Breeds represented included Golden Retrievers (6), Labrador Retrievers (5), mixed breed dogs (3), German Shepherds (3), Australian Shepherds (2), and 1 each of the following breeds: German Shorthaired Pointer, Irish Wolfhound, Pembroke Welsh Corgi, Rhodesian Ridgeback, Standard Poodle, Weimaraner, Wheaton Terrier, Boxer, and Dachshund. An additional 28 healthy age- and breed-matched control dogs were also enrolled. The breed distribution of the controls was the same as the SHP dogs with the exception of the Irish Wolfhound. An age-matched dog could not be located, so a Great Dane of the same age was recruited. Per study design, there were no significant differences between the SHP dogs and the controls in age (10.4 ± 1.7 vs. 10.3 ± 2.0 y, $P = 0.829$) or in sex distribution (female 32%, male 68% vs. female 50%, male 50%, $P = 0.28$).

Of the 28 SHP dogs recruited, 22 were sent to surgery and 6 were euthanized shortly after hospital admission. Of the 22 dogs that went to surgery, 18 survived, 3 were euthanized at surgery or in the postoperative period, and 1 died in the postoperative period. Histopathology was available in all 28 cases and the following diagnoses were obtained: hemangiosarcoma (20, 71.4%), hepatocellular carcinoma (3, 10.7%), and 1 each of benign hematoma, adrenal carcinoma, prostatic carcinoma, histiocytic sarcoma, and splenic soft tissue sarcoma.

Coagulation

Results of platelet count, coagulation screening tests, Fg, antithrombin, and d-dimer for each group of dogs are summarized in Table 1. Mean Fg concentration was not significantly different between the SHP and control dogs; however, mean PT and aPTT were significantly longer and platelet counts lower in SHP dogs. A significantly higher percentage of SHP dogs had prolongation of the aPTT beyond the reference interval than control dogs (29% vs. 0%, $P = 0.008$), and more SHP dogs than controls were hypofibrinogenemic (25% vs. 0%) or hyperfibrinogenemic (14.3% vs. 0%, $P = 0.001$), but there was no difference between SHP dogs and controls in the percentage with prolonged PT (7.1% vs. 0%, $P = 0.49$). Platelet counts were adequate in all control dogs, but counts were low or blood smears were assessed as thrombocytopenic in 17 (60.7%) of the SHP dogs. Platelet clumping prevented accurate counts in 2 control dogs and 4 SHP dogs, but among dogs for which platelet counts could be obtained, SHP dogs had significantly lower counts than controls (see Table 1). There was evidence of AT deficiency in some SHP dogs, with decreased AT documented in 21.4% of cases. There was also evidence of protein C deficiency with values below the reference range in 39.3% of SHP cases. Stepwise linear regression analysis showed that of protein C activity, Fg concentration, platelet count, AT, and plasma lactate concentration, only protein C activity was independently associated with aPTT ($r = -0.73$, $P < 0.0001$) and PT ($r = -0.69$, $P = 0.0002$), with decreasing protein C activity associated with increases in clotting times (Figure 1).

Among the TEG coagulation parameters for the citrated native samples, only MA differed between the groups, with control dogs having larger MA than SHP dogs (see Table 2). Multiple linear regression analysis

Table 1: Results of platelet count, coagulation tests, fibrinogen, antithrombin, protein C, and d-dimer assays for 28 dogs with spontaneous hemoperitoneum and results of coagulation screening tests in 28 control dogs

Group	PT (sec)	aPTT (sec)	Fibrinogen (μmol/L; mg/dL)	AT (%)	Protein C (%)	d-Dimers (nmol/L; ng/mL)	Platelets ×10³/μL
Control	11.6 ± 0.577	12.5 ± 1.56	9.17 ± 2.23; 312 ± 76.0	N/A	N/A	N/A	261 (164–393)
SHP	13.0 ± 1.76*	16.2 ± 4.21*	8.70 ± 6.56; 296 ± 223	75 ± 18.8	84.8 ± 33.4	7,440 ± 5,202; 2,144 ± 1,499	104 (23–421)§
Reference interval	11–15.5	10–17	4.41–14.4; 150–490	65–145	75–135	0–1,562; 0–450	186–545

Normally distributed data are presented as mean ± standard deviation and nonnormally distributed data are presented at median and range.
*Significantly different from controls, independent samples t-test, $P < 0.0002$; §significantly different from controls, Mann–Whitney U-test, $P < 0.0001$. The coagulation assay reference intervals were provided by the diagnostic laboratory[c] and were determined from a group of 25 healthy dogs. aPTT, activated partial thromboplastin time; AT, antithrombin; N/A, not available; PT, prothrombin time; SHP, spontaneous hemoperitoneum.
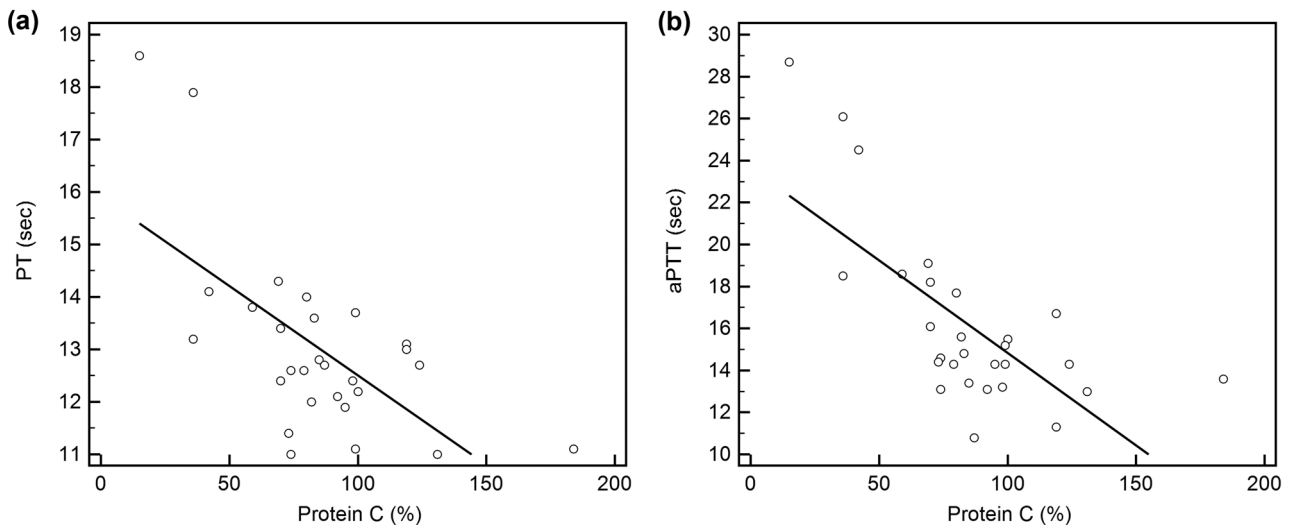
Figure 1: In dogs with spontaneous hemoperitoneum (SHP), the (a) prothrombin time (PT) and (b) activated partial thromboplastin time (aPTT) linearly increased with decreasing protein C activity (linear regression, $P < 0.001$).

showed that of Fg concentrations, platelet counts, and group (SHP vs. control), both Fg concentration ($r = 0.70$, $P < 0.0001$) and platelet count ($r = 0.44$, $P = 0.0018$) were independently associated with MA but group was not. In addition, among the SHP dogs, multiple linear regression analysis showed that of Fg concentration, platelet count, protein C activity, and plasma lactate concentration, only Fg was independently associated with MA ($r = 0.76$, $P < 0.0001$). None of the standard TEG coagulation parameters differed between the groups with 50 U/mL of tPA added (see Table 3).

Fibrinolysis
With the addition of 100 U/mL of tPA, there was no TEG clot formation in 17/56 (30%) of the dogs in the study. Therefore, TEG data were only analyzed for the native and 50 U/mL tPA samples.

Among the SHP dogs, there was evidence of ongoing fibrinolysis in the majority with increased d-dimer concentration in 27/28 cases (2,144 ± 1,499 ng/mL;

reference range = 25–245 ng/mL). However, linear regression analysis showed no association between d-dimer concentrations and the degree of shock as reflected by plasma lactate concentration ($P = 0.23$). Minimal fibrinolysis was noted in TEG analyses of citrated native blood samples within 60 minutes of MA in both the controls and the SHP dogs and there was no difference in LY30 or LY60 between the groups (Table 2). However, in reaction mixtures containing 50 U/mL of tPA, fibrinolysis was evident at 30 and 60 minutes after MA was reached in all dogs, and both TEG LY30 measured with the addition of 50 U/mL of tPA to the blood sample (LY30$_{50}$) and TEG LY60 measured with the addition of 50 U/mL of tPA to the blood sample (LY60$_{50}$) were significantly greater in SHP dogs than in control dogs (Table 3, Figure 2). Protein C activity was high in 1 SHP dog and low in 11 (39.3%) SHP dogs. Linear regression analysis showed no statistically significant association between protein C concentrations and severity of shock as reflected in lactate

Table 2: Tissue factor activated thromboelastography parameters for calcified citrated whole blood samples for 28 dogs with spontaneous hemoperitoneum and 28 control dogs

Group	R (min)	K (min)	Angle (°)	MA (mm)	LY30 (%)	LY60 (%)
Control	2.8 (1.4–4.0)	3.0 (0.80–5.30)	54.5 ± 12.0	51.1 ± 6.8	0 (0–5.7)	1.7 (0–13.0)
SHP	2.5 (1.7–8.2)	3.25 (1.0–11.8)	49.5 ± 18.8	42.5 ± 15.2*	0 (0–23.1)	1.10 (0–47.4)

Normally distributed data are presented as mean ± standard deviation, while nonnormally distributed data are presented as median (range).
*Significantly different from controls, independent samples *t*-test, $P = 0.0081$. MA, maximum amplitude; LY30, lysis at 30 minutes; LY60, lysis at 60 minutes; SHP, spontaneous hemoperitoneum.

Table 3: Tissue factor activated thromboelastography parameters for calcified citrated whole blood samples for 28 dogs with spontaneous hemoperitoneum dogs and 28 control dogs with 50 U/mL of tissue plasminogen activator added to the reaction mixture to accelerate fibrinolysis

Group	R_{50}	K_{50}	$Angle_{50}$	MA_{50}	$LY30_{50}$	$LY60_{50}$
Control	2.4 (1.4–3.9)	4.7 ± 3.2	49.6 ± 13.6	41.9 ± 11.1	31.2 ± 19.5	54.3 ± 20.0
SHP	2.2 (1.3–8.4)	4.2 ± 3.4	50.2 ± 19.2	35.3 ± 15.8	48.1 ± 32.2*	67.1 ± 24.4*

Normally distributed data are presented as mean ± standard deviation, while nonnormally distributed data are presented as median (range).
*Significantly different from controls, independent samples *t*-test, $P < 0.05$. SHP, spontaneous hemoperitoneum; R_{50}, TEG R time measured with the addition of 50 U/mL of tPA to the blood sample; K_{50}, TEG K time measured with the addition of 50 U/mL of tPA to the blood sample; $angle_{50}$, TEG angle measured with the addition of 50 U/mL of tPA to the blood sample; MA_{50}, TEG MA measured with the addition of 50 U/mL of tPA to the blood sample; $LY30_{50}$, TEG LY30 measured with the addition of 50 U/mL of tPA to the blood sample; $LY60_{50}$, TEG LY60 measured with the addition of 50 U/mL of tPA to the blood sample.
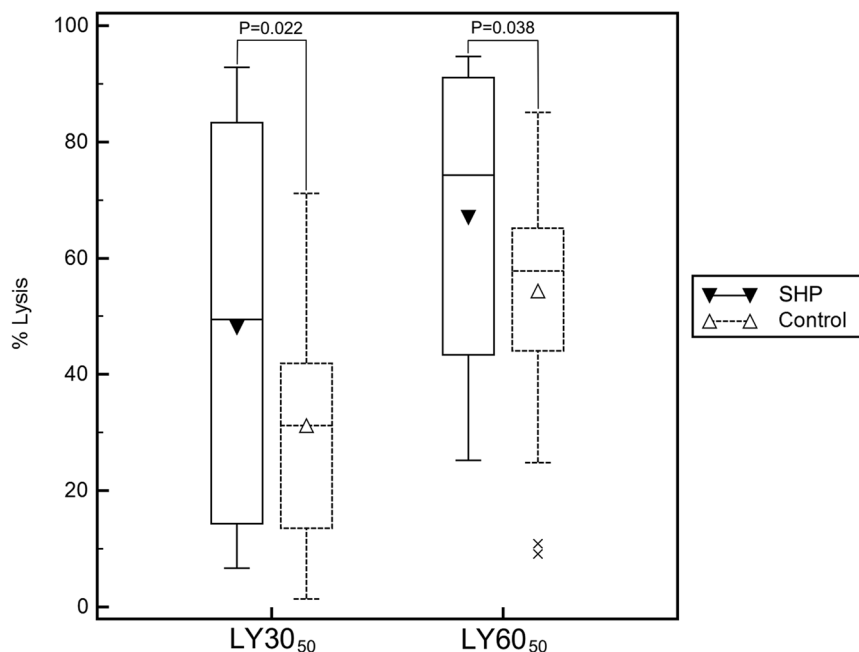

Figure 2: Dogs with spontaneous hemoperitoneum (SHP) demonstrated hyperfibrinolysis compared to control dogs with significant increases in both $LY30_{50}$ and $LY60_{50}$ (Student's *t*-test). Box and whisker plots, triangle = mean, central line = median, box = 25–75 percentile, error bars = range excluding outside values, X = outside value smaller than lower quartile minus 1.5 times the interquartile range. $LY30_{50}$, lysis at 30 minutes with 50 U/mL tPA added; $LY60_{50}$, lysis at 60 minutes with 50 U/mL tPA added.

Stepwise multiple linear regression analysis showed that of lactate, protein C, PT, aPTT, d-dimers, AT, and PCV, only lactate concentration was significantly associated with $LY30_{50}$ ($r = 0.59$, $P = 0.01$; Figure 3) and $LY60_{50}$ ($r = 0.52$, $P = 0.01$). Figure 4 shows TEG data from 2 individual SHP dogs, 1 with low lactate on presentation and 1 with high lactate on presentation.

Of the 18 dogs sent to surgery and ultimately discharged from the hospital, 12 (67%) received packed red blood cells (median dose = 11.6, range = 5.2–38.1
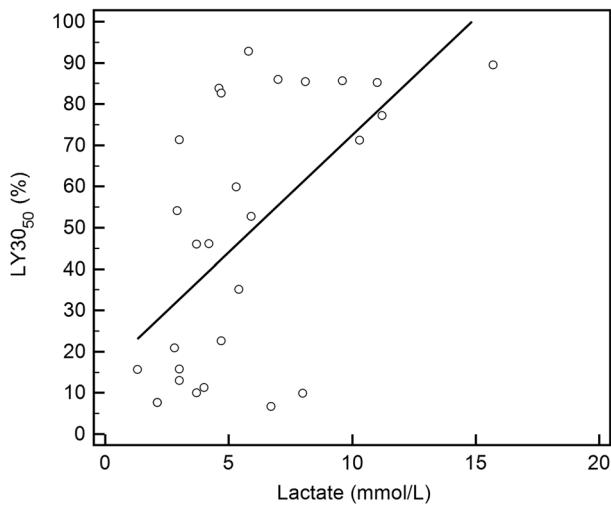
Figure 3: In dogs with spontaneous hemoperitoneum, the degree of fibrinolysis at 30 minutes after MA ($LY30_{50}$) increases linearly with increasing plasma lactate concentration (linear regression, $P = 0.0008$). MA, maximum amplitude.

mL/kg) and 5 (28%) received fresh frozen plasma (median dose $= 7.7$, range $= 6.5$–15.2 mL/kg). Stepwise multiple linear regression analysis also showed that among these 18 surviving dogs, of $LY30_{50}$, protein C, PT, aPTT, Fg, AT, d-dimers, and admission PCV only $LY30_{50}$ was significantly associated with the dose of fresh frozen plasma administered during hospitalization, with increased plasma administered as admission $LY30_{50}$ increased ($r = 0.50$, $P = 0.03$; Figure 5), but none of the variables were significantly associated with the dose of packed red blood cells administered. The 10 dogs that were euthanized or died were excluded from this analysis since their potential transfusion requirements during treatment could not be predicted.

Discussion

We found that some dogs with SHP demonstrate evidence of hypocoagulability and hyperfibrinolysis at hospital admission, that the severity of these abnormalities
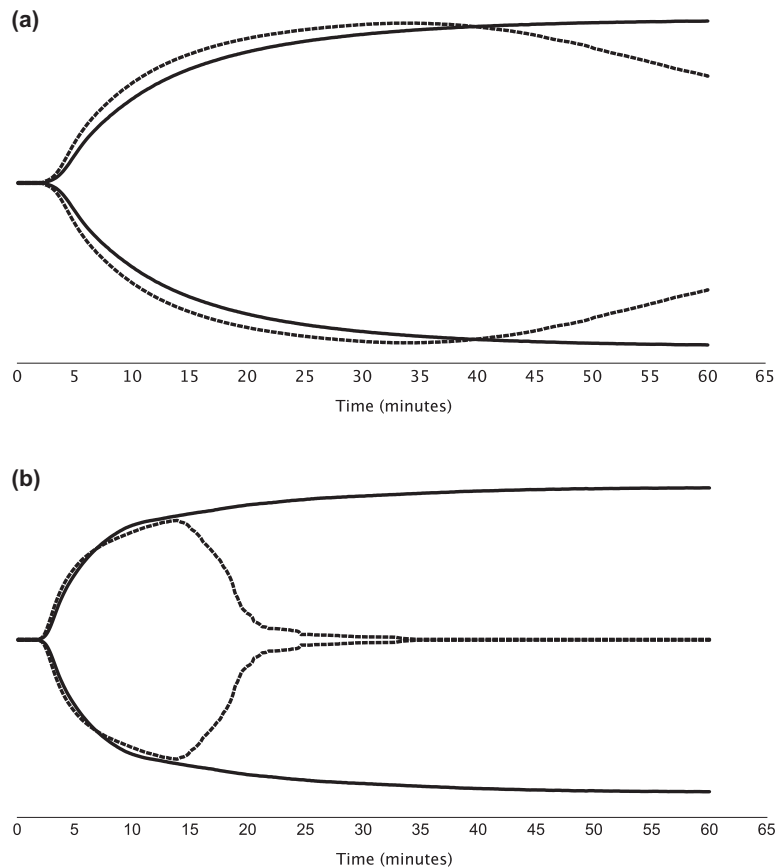

Figure 4: Standard thromboelastography (TEG) tracing (solid line) and TEG tracing with 50 U/mL of tissue plasminogen (tPA) added to the reaction mixture (dashed line) for 2 dogs with spontaneous hemoperitoneum (SHP). (a) Low plasma lactate $= 2.1$ mmol/L showing minimal fibrinolysis, (b) high lactate $= 11.0$ mmol/L showing marked fibrinolysis with added tPA. Minimal fibrinolysis was noted in standard TEG tracings for either patient.
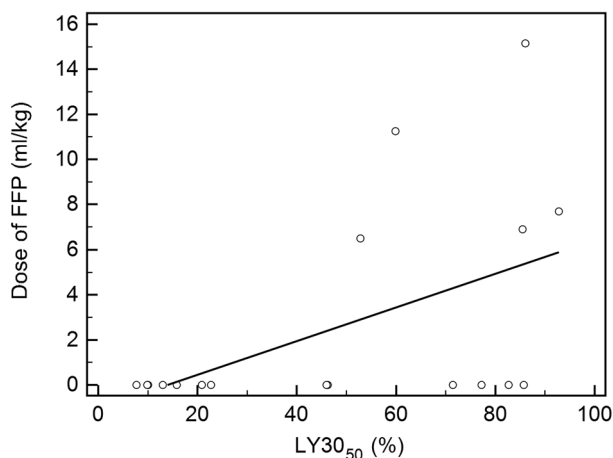
Figure 5: In dogs with spontaneous hemoperitoneum that survived to discharge ($N = 18$), the dose of fresh frozen plasma (FFP) administered increased linearly with the degree of fibrinolysis at 30 minutes after MA ($LY30_{50}$), but was not associated with protein C, PT, aPTT, fibrinogen, AT, d-dimers, or admission PCV (multiple linear regression, $P = 0.033$). aPTT, activated partial thromboplastin time; AT, antithrombin; $LY30_{50}$, lysis at 30 minutes with 50 U/mL tPA added; PCV, packed cell volume; PT, prothrombin time.

is associated with the severity of shock as assessed by plasma lactate concentrations, and that the severity of the coagulation abnormalities are also associated with protein C deficiency. These findings are similar to the human syndrome of ACoTS. This complex coagulopathy is believed to be mediated by activation of the protein C pathway as a consequence of combined tissue injury and shock. In response to hypoxia and hypoperfusion, thrombomodulin expression is upregulated on endothelial cells resulting in increased binding and transformation of protein C to its enzymatically active form, activated protein C (aPC). In addition to aPC's anticoagulant effect, aPC inactivates PAI-1 and thrombin activatable fibrinolysis inhibitor and thereby dis-inhibits fibrinolysis.[1–4] The recognition that hyperfibrinolysis is an important cause of hemorrhage in ACoTS suggests that treatment of SHP dogs with antifibrinolytic agents such as epsilon aminocaproic acid and tranexamic acid may be of benefit as has been found in large ACoTS treatment trials.[25]

Dogs with SHP demonstrated many laboratory criteria of hypocoagulability at admission compared to age- and breed-matched control dogs as evidenced by low platelet count, prolonged clotting times, and low MA. Multiple linear regression analysis showed that lower Fg concentrations and platelet counts likely accounted for the lower MA in the SHP dogs rather than some other factor associated with the hemoperitoneum, since presence of SHP was not a significant, independent predictor

of MA when the model also contained platelet counts and Fg concentrations. This is consistent with previous studies documenting the influence of platelet count and Fg on MA.[26–28] Multiple linear regression analysis also showed that among SHP dogs, Fg was an independent predictor of MA, while protein C, lactate, and platelet counts did not affect MA.

The enhanced generation of aPC in the early phase of ACoTS results in depletion of circulating levels of its precursor zymogen, protein C.[1–4] Direct measurements of plasma aPC are difficult to perform due to its cell-surface generation and action and short (15 min) plasma half-life. Most clinical studies, therefore, measure plasma protein C in functional assays after in vitro activation.[29] The protein C activity assay used in our study was configured with a snake venom reagent that is a specific and rapid activator of protein C. The quantity of active enzyme thus formed is detected by its cleavage of a synthetic peptide substrate (CBS 42.46) and resultant color change of the assay mixture.[k] Our data show a strong independent, linear association between decreasing protein C activity and prolongation of both PT and aPTT. Although clotting times in the SHP dogs were relatively prolonged compared with controls, few had values that fell outside reference ranges. This finding indicates adequate hepatic function for coagulation factor and protein C synthesis, and suggests that the cause of protein C deficiency in SHP dogs was activation and subsequent depletion.

Fibrinolysis was also documented in the SHP dogs, with 27/28 dogs demonstrating increased d-dimers. Because the underlying disease in these dogs was massive intraabdominal bleeding with activation of coagulation at the source of hemorrhage, high circulating d-dimer concentration may reflect normal lysis secondary to increased fibrin formation rather than a primary pathologic up-regulation of the fibrinolytic system. The LY30 and LY60 TEG parameters assess fibrinolytic potential by quantifying the degree of clot lysis over time in whole blood. This assay is limited by the absence of local contributors to fibrinolysis such as endothelial cells and blood cell and platelet responses to endothelial damage, and as such, these in vitro fibrinolysis parameters may not reflect in vivo fibrinolytic potential in areas of endothelial damage.[30,31] Citrated native TEG LY30 and LY60 showed minimal measurable fibrinolysis by 60 minutes post MA. The addition of 50 U/mL of tPA resulted in measurable fibrinolysis in all dogs, while 100 U/mL of tPA resulted in poor initial clot formation in many dogs, minimizing the utility of the higher tPA concentration. Enhancement of in vitro fibrinolysis with 50 U/mL of tPA increased the ability of the TEG assay to identify differences in fibrinolysis (documented as increased $LY30_{50}$ and $LY60_{50}$) between SHP

and control dogs. In contrast to the increased d-dimer concentrations, the increased $LY30_{50}$ and $LY60_{50}$ suggest an increase in fibrinolytic capacity and activity rather than fibrin degradation as a secondary response to enhanced fibrin formation. Multiple linear regression analysis showed that the enhanced fibrinolysis was independent of changes in coagulation activity or factors, suggesting that this disorder occurs independently of coagulopathy and could potentially represent a novel therapeutic target in patients with SHP. Clinical trials will be necessary to determine if antifibrinolytic agents may be of use in these patients. It should be noted, as mentioned above, that systemic measures such as whole blood $LY30_{50}$ do not fully characterize enhanced fibrinolysis at the local endothelial level. The use of antifibrinolytic drugs for patients at risk of enhanced fibrinolysis (such as patients with severe trauma and perioperative bleeding) is currently recommended in human medicine based on the results of large scale clinical trials, regardless of laboratory documentation of hyperfibrinolysis, and it is likely that a similar approach will be necessary in veterinary medicine.[32,33]

As we hypothesized, increasing lactate concentration, possibly indicating worsening shock, was linearly associated with increased fibrinolytic potential as represented by the $LY30_{50}$ and $LY60_{50}$. However, in contrast to the coagulation parameters, there was no statistically significant relationship between protein C activity and $LY30_{50}$ or $LY60_{50}$. We did not directly measure aPC, PAI-1, and other fibrinolytic pathway proteins to further define the underlying mechanisms of hyperfibrinolysis in the SHP dogs. These mechanisms may differ between SHP cases and human trauma patients and/or we may not have had enough patients in this study to identify an association between protein C levels and fibrinolytic activity. Future studies with more dogs and evaluation of additional fibrinolytic pathway mediators are needed to fully elucidate the mechanism of hyperfibrinolysis in dogs with SHP.

Although the hypocoagulability in SHP dogs at admission was not associated with the amount of blood products used in these patients (and primary clinicians were unaware of the results of the coagulation testing performed herein), the severity of hyperfibrinolysis (as measured by $LY30_{50}$) at admission was associated with the total dose of plasma administered during hospitalization. This pattern has also been observed in human trauma patients, with hyperfibrinolytic patients (documented on TEG analysis) being more likely to require higher doses of FFP to treat worsening coagulopathies compared to patients without hyperfibrinolysis at admission.[34] Documentation of hyperfibrinolysis at admission in dogs with SHP may be useful for identifying patients more likely to be at risk of refractory hemorrhage during treatment,

which could have an impact on cost of treatment and prognosis. We were not able to demonstrate an association between either hyperfibrinolysis or coagulopathy and red blood cell transfusions in this cohort of dogs with SHP. It should be noted that of the 28 SHP dogs in this study, only 18 survived to discharge and were included in the blood product usage analysis. Of those 18, only 5 received plasma transfusions, and our finding of an association between $LY30_{50}$ at admission and dose of plasma administered should be interpreted in light of this small number of cases. It should also be noted that a larger number of dogs (12) received packed red blood cell transfusions, and no association between admission $LY30_{50}$ and red blood cell transfusion was found. Because of this very low number of cases, these results should be interpreted cautiously. Further studies of blood product usage in a larger cohort of animals with better defined transfusion criteria are needed to more fully investigate the relationship between hyperfibrinolysis at admission and red blood cell and plasma requirements and to determine if our finding of an association between admission $LY30_{50}$ and plasma transfusions is truly indicative of an association between admission $LY30_{50}$ and bleeding tendencies, or simply a statistical anomaly due to the small sample size.

In 27/28 (96%) of the SHP dogs in this study, histopathology showed the underlying cause of SHP to be malignant neoplasia. Previous studies have reported a lower prevalence of malignancy of 70–80%.[35–37] The reason for this difference is unclear, but it is possible that the hemostatic abnormalities identified in this study may not generalize to animals with other causes of hemoperitoneum (eg, trauma). Because only 1 patient in the study had nonmalignant disease, comparisons between the hemostatic derangements in animals with malignant and nonmalignant disease were not possible. Vascular neoplasms such as hemangiosarcoma can also have independent effects on fibrin generation, and future studies are warranted to better characterize hemostasis in patients with benign SHP and other causes of acute, severe blood loss.[38] Until such data are available, we cannot rule out the possibility that the differences in fibrinolysis between the control group and the SHP dogs were due to the underlying malignant disease rather than to the effects of tissue injury and shock. However, this would not explain the association between the degree of hyperfibrinolysis and lactate concentrations (our indicator of shock severity), suggesting that these differences are unlikely due solely to the malignant process.

Although TEG is increasingly being used to provide a global evaluation of hemostasis (including both coagulation and fibrinolysis) in veterinary patients, concerns about poor reproducibility and high interoperator and interlaboratory variability remain. An

international working group is currently attempting to standardize the approach to human TEG analysis to improve interlaboratory variation, although their first study using kaolin-activated TEG analysis of platelet-rich plasma samples showed interlaboratory coefficients or variation of greater than 10% for some parameters.[39] A recent study using rotational thromboelastometry, a technology very similar to TEG, showed that citrated whole blood samples run using the same protocol at multiple institutions had acceptable coefficients of variation, with the best results obtained when either celite or TF activation was used.[40] This is consistent with the findings of a study in dogs showing good reproducibility when citrated blood samples were activated using high concentrations of TF for activation.[23] Because the institutions involved in the current study are geographically separated by large distances, we were unable to specifically evaluate interlaboratory coefficients of variation for whole blood TEG parameters. In addition, the distribution of the control patients and SHP patients recruited from each institution was not the same, leading to the possibility that the differences in fibrinolysis parameters between control dogs and SHP dogs in this study may be spurious. Because our assay used citrated whole blood with a high concentration of TF (1:3,600 dilution) and were run by a single person at each institution using a well-defined protocol, we are optimistic that interlaboratory variability was minimal, but future studies of the interlaboratory variability of fibrinolysis parameters in canine whole blood are warranted, and this limitation should be recognized when interpreting the results of this study.

This study has a number of additional limitations. Coagulation and fibrinolysis testing was only done at a single time point, on presentation to the emergency department. Because of the nature of this disease, it was not possible to know when bleeding started in these patients, and it is likely that there was variability in the duration of bleeding before presentation in these patients. We did not evaluate coagulation and fibrinolysis at later time points during surgery and recovery, so changes in these parameters over time and time to resolution cannot be determined. Criteria for administration of blood products were not standardized as they were at the discretion of the attending clinician, so our blood product usage findings should be interpreted cautiously. Among the 10 dogs with SHP that died, 9 were euthanized at initial diagnosis or at surgery due to gross evidence of metastatic disease. Due to the high prevalence of euthanasia among the nonsurvivors, the effect of admission coagulopathy and hyperfibrinolysis on mortality could not be investigated. Finally, we chose plasma lactate concentration as our marker of the degree of shock in this study. There is evidence in people that lactate is a useful prognostic indicator in hemorrhagic shock due to ruptured AAA.[41] However, lactate production can increase independently of anaerobic metabolism.[42] A single parameter is unlikely to fully characterize the severity of a complex pathophysiologic state like shock, and although composite parameters including physiologic parameters such as heart rate and blood pressure have been proposed, there is limited data regarding the utility of these indices in people or dogs.[43–45] Ultimately, our conclusions regarding the associations between hemostatic derangements and severity of shock must be interpreted cautiously in light of the absence of a robust measure of shock severity.

In spite of these limitations, we found that dogs with SHP have complex hemostatic abnormalities, with some patients demonstrating features of hypocoagulability and hyperfibrinolysis, and that these hemostatic defects appear to be associated with the severity of shock as reflected in blood lactate concentrations. This suggests that treatment with antifibrinolytic drugs could be useful to reduce blood loss in these patients, representing an inexpensive alternative to blood products. Further studies of nonmalignant acute hemorrhagic syndromes and more detailed pharmacokinetic, and pharmacodynamic, evaluation of antifibrinolytic drugs in dogs are warranted. Ultimately, a prospective trial evaluating the efficacy and safety of antifibrinolytic drugs in dogs with SHP is needed to determine whether pharmacologic manipulation of fibrinolysis improves outcomes in this patient population.

Footnotes

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