Journal of Veterinary Emergency and Critical Care

Original Study

Journal of Veterinary Emergency and Critical Care **23**(1) 2013, pp 14–22
doi: 10.1111/vec.12013

Alterations in the hemostatic profiles of dogs with naturally occurring septic peritonitis

Adrienne M. Bentley, DVM, DACVS; Philipp D. Mayhew, BVMS, DACVS; William T. N. Culp, VMD, DACVS and Cynthia M. Otto, DVM, PhD, DACVECC

Abstract

Objective – To characterize derangements in the hemostatic profiles of dogs with naturally occurring septic peritonitis and determine if such derangements were predictive of survival.
Design – Prospective, observational single cohort study.
Setting – University veterinary teaching hospital.
Animals – A total of 27 client-owned dogs with naturally occurring septic peritonitis.
Interventions – Standard treatment included fluid resuscitation, antimicrobial therapy, supportive care, and surgery provided at the discretion of the primary clinician. Blood was collected preoperatively and on days 1 and 3 postoperatively for platelet count, prothrombin time, activated partial thromboplastin time, D-dimer and fibrinogen concentrations, total protein C (PC) and antithrombin (AT) activities, and thromboelastography.
Measurements and Main Results – Sixteen of 27 (59%) dogs survived. Preoperative PC deficiency was identified in 10 of 11 (91%) nonsurvivors and 2 of 15 (13%) survivors. Preoperative AT deficiency was identified in 10 of 11 (91%) nonsurvivors and 14 of 15 (93%) survivors. Compared to survivors, nonsurvivors had lower mean preoperative PC (98 ± 24% versus 49 ± 26%; $P < 0.001$) and AT (53 ± 9% versus 32 ± 16%; $P < 0.001$) activities. Anticoagulant activities decreased on day 1 postoperatively. As a predictor of survival, preoperative PC activity of more than 60% achieved a sensitivity of 93% and specificity of 82%. Preoperative AT activity of more than 41.5% achieved a sensitivity of 100% and specificity of 82%. The maximum amplitude, α angle, and coagulation index from preoperative thromboelastograms of survivors were significantly greater (more hypercoagulable) than nonsurvivors ($P < 0.01$), with the maximum amplitude being the most specific predictor of survival (100%).
Conclusions – Deficiencies of PC and AT and hypercoagulability appear to be consistent features of naturally occurring canine sepsis and may be useful prognostic indicators in canine septic peritonitis.

(J Vet Emerg Crit Care 2013; 23(1): 14–22) doi: 10.1111/vec.12013

Keywords: antithrombin, protein C, secondary peritonitis, sepsis, thromboelastography

Introduction

Bacterial peritonitis is a common cause of sepsis in dogs and is associated with high mortality.[1–3] Reported survival rates for dogs with septic peritonitis vary from 27% to 71%, with most studies reporting approximately 50% survival.[4–10] One retrospective study reported 60% survival among dogs treated surgically for septic

Abbreviations

aPTT	activated partial thromboplastin time
AT	antithrombin
CBC	complete blood count
CI	coagulation index
MA	maximum amplitude
PC	protein C
POC	point-of-care
PT	prothrombin time
ROC	receiver operator curve
SIRS	systemic inflammatory response syndrome
TEG	thromboelastography

From the Department of Clinical Studies, Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA 19104.
Dr. Bentley's current address: Veterinary Medical and Surgical Group, 2199 Sperry Avenue, Ventura, CA 93003.
Dr. Mayhew's and Dr. Culp's current address: Department of Surgical and Radiological Sciences, University of California, Davis, CA 95616.

Financial support provided by The Barry and Savannah French-Poodle Memorial Fund and the Department of Clinical Studies, Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania.

The authors declare no conflicts of interest.

Presented in part at the 2007 American College of Veterinary Surgeons Veterinary Symposium, Chicago, Illinois, October 17–21, 2007.

Address correspondence and reprint requests to
Dr. Cynthia Otto, Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, 3900 Delancey St, Philadelphia, PA 19104, USA.
Email: cmotto@vet.upenn.edu
Submitted December 5, 2011; Accepted November 25, 2012.

© Veterinary Emergency and Critical Care Society 2013

peritonitis with no detectable improvement in survival rate over a 16-year period.[11] Death in dogs treated for septic peritonitis typically results from complications such as refractory hypotension, disseminated intravascular coagulation, and multiple organ failure, often in the immediate postoperative period.[5,7,9]

Identification of prognostic indicators in clinical cases of canine septic peritonitis remains challenging. Few of the many physical and laboratory findings examined retrospectively have been associated with survival. In 2 separate studies, survivors had higher blood pressure, including blood pressure measured on admission, intra- and postoperatively.[7,11] Higher preoperative serum albumin has also been associated with survival in clinical cases of canine septic peritonitis.[11] Prospective clinical studies evaluating biomarkers specific for canine septic peritonitis are lacking.

In people with sepsis, multiple studies have shown an association between deficiencies in protein C (PC) and antithrombin (AT) and mortality.[12–15] Among surgical and nonsurgical patients with sepsis, both baseline and sustained PC deficiency have been associated with mortality.[13,16] In people with septic peritonitis, AT was lower among nonsurvivors from postoperative day 3. In the same population of patients, PC activity was lower among nonsurvivors at every point of sample collection, including preoperatively.[13]

Similar to the heterogeneity of clinical signs and sources of sepsis in people, naturally occurring canine sepsis represents a highly heterogeneous population. In a population of 20 septic dogs with heterogeneous sources of sepsis, PC and AT activities at the time of hospital admission were lower than in normal dogs, but neither PC nor AT was associated with survival.[2] Serial evaluation of PC and AT in 12 septic dogs showed changes in activities of both anticoagulants over time and higher PC activity in survivors on day 2.[3] One of the most common sources of sepsis in dogs is septic peritonitis.[1–3] Dogs with septic peritonitis may constitute a more homogeneous population of septic dogs since they typically share a similar endogenous bacterial source of infection (most commonly enteric bacteria), a similar diagnostic approach (imaging and abdominal fluid evaluation), and a similar therapeutic intervention (source control).

The primary goal of this study is to characterize alterations of hemostasis in a relatively homogeneous population of septic dogs, those undergoing surgical treatment of naturally occurring septic peritonitis. The secondary goal of this study is to determine the prognostic significance of various coagulation parameters in this population of dogs using both routine and more specialized laboratory tests.

Materials and Methods

Patient selection

The study protocol was approved by the Institutional Animal Care and Use Committee and the Privately Owned Animal Protocol Committee. Client-owned dogs that were admitted to the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania and diagnosed with septic peritonitis based on the presence of intracellular bacteria on cytology of abdominal effusion or suspicion of peritonitis confirmed by gross evidence of gastrointestinal perforation at surgery or positive bacterial culture of abdominal effusion were eligible for inclusion. Additional inclusion criteria included owner's informed signed consent for study enrollment and surgical treatment and a minimum body weight of 5 kg. Treatment of enrolled dogs was determined by the primary clinician and was not standardized. Dogs receiving anticoagulant therapy or a transfusion of any blood product prior to collection of the initial blood sample were excluded. Administration of an anticoagulant to an enrolled patient according to the primary clinician necessitated exclusion of that patient from the remainder of the study protocol.

Sampling procedures and assays

The first blood sample (day 0) was collected prior to surgery and subsequent samples were collected on postoperative days 1 and 3, either by direct venipuncture or through an existing vascular catheter after a presample volume was collected. Laboratory tests performed at each of the 3 sampling points included a routine coagulation panel (platelet count, prothrombin time [PT], and activated partial thromboplastin time [aPTT]), D-dimer and fibrinogen concentrations, total PC and AT activities, and thromboelastography (TEG). A CBC and serum biochemistry profile were also performed as a part of the study protocol prior to surgery.

All samples were analyzed at the time of collection except for the AT and PC activities and fibrinogen concentration. All serum biochemistry analytes were measured on a chemistry analyzer,[a] and CBCs were performed using optical light scatter analysis.[b] Coagulation data were either measured via clotting and immunoturbidometric assays[c] using reagents from the same manufacturer[d] or via an automated point-of-care (POC) coagulation analyzer.[e] The percent prolongation in PT or aPTT was calculated by the difference between the patient value and the upper range of normal divided by the upper range of normal for the machine used. TEG[f] was performed on recalcified nonactivated citrated blood according to the laboratory standard methods, which have been previously reported.[17–19] The reported coagulation

index (CI) was calculated from the measured values of *R*, *K*, maximum amplitude (MA), and α angle by a proprietary algorithm integrated in the manufacturer-supplied software.[f] In people, this parameter has been validated as an overall assessment of coagulation status.[20,21] While this parameter has been reported previously in dogs,[18] it has never been formally validated in dogs.

Citrated plasma was stored at −70°C and submitted in batches to the Cornell University Diagnostic Laboratory for determination of the anticoagulant activities and fibrinogen concentration. Plasma AT and PC activities were measured using synthetic chromogenic substrate kits.[g] The assays' standard curves were derived based on dilutions of pooled normal canine plasma. The AT and PC of test plasmas are reported as the percentage of the canine standard, which has an assigned value of 100% activity. The fibrinogen concentration was determined by the Clauss method using a 100 NIH Units/mL human thrombin reagent.[h]

Other data recorded

Patient breed, age, weight, and neuter status were recorded. Dogs met criteria for systemic inflammatory response syndrome (SIRS) if they met at least 2 of the following 4 criteria: heart rate >140/min, respiratory rate >20/min, temperature >39.4°C (102.9°F) or <37.8°C (100.0°F), or WBC count $>16 \times 10^9$/L or $<6 \times 10^9$/L ($>16 \times 10^3/\mu$L or $<6 \times 10^3/\mu$L).[22] Surgical findings including the source of sepsis, type of closure and drain, and results of bacterial cultures of abdominal effusion were recorded. Survival to discharge and cause of death or reason for euthanasia were recorded as outcome parameters. Owners were contacted by phone to determine the patient's status 30 days after surgery if the status was not evident from the medical record.

Statistical analysis

To compare data between survivors (survival to discharge) and nonsurvivors (died or euthanized during hospitalization) or to compare proportional data between different days among the survivors, the Fisher's exact test or the Chi-square test was used for categorical variables. Continuous variables were assessed for normality using the Shapiro–Wilk W test. The *t*-test and the Mann–Whitney rank sum test were used to compare continuous variables between survivors and nonsurvivors with normal and non-normal distributions, respectively. Receiver operator curves (ROCs) and associated sensitivity and specificity data for cut-off values were generated to analyze data for days 0 and 1. Normally distributed serial data for survivors was analyzed using a one-way repeated measures analysis of variance with pairwise multiple comparisons using the Holm–Sidak method. Serial data for survivors that failed normality was tested by the Friedman repeated measures analysis of variance on ranks with post hoc Tukey testing; only patients with complete data sets were able to be analyzed by this method. Categorical data are presented as frequencies and percentages, and continuous data are presented as means ± standard deviation (SD) or medians with interquartile range for normal and non-normal distributions, respectively. All analyses were performed using statistical software.[i] Statistical significance was set at $P < 0.05$. A correction to the *P* values for multiple comparisons was not applied.[23,24]

Results

Survival

Fifty-one dogs presented to the teaching hospital with a diagnosis of septic peritonitis between February 2006 and May 2008. Of those cases, 10 were ineligible because surgical treatment was declined (due to financial constraints or poor prognosis), and 14 were not enrolled due to failure to notify the investigators of the eligible case. Twenty-seven dogs met the inclusion criteria and were enrolled in the study.

Of the 27 cases enrolled, anticoagulant therapy was initiated in 2 dogs postoperatively after collection of day 1 samples, necessitating exclusion from day 3 sampling, and 2 survivors were discharged before collection of the day 3 postoperative samples. Day 0 D-dimer data were not available for cases enrolled after regular laboratory hours since a POC analyzer for this parameter was not available. In some instances, a quantitative platelet count was not available due to platelet clumping, and laboratory or machine error accounted for loss of certain coagulation data in other cases. Finally, in some cases enrolled after regular laboratory hours, there was not an investigator available to process a sample for day 0 TEG data.

Sixteen of 27 (59%) dogs survived to discharge, and 11 dogs died or were humanely euthanized at the request of their owners. Of the survivors, 15 of 16 dogs that survived to discharge were still alive 30 days after discharge; one dog was euthanized for thoracolumbar myelopathy due to intervertebral disc protrusion within 30 days of discharge from the hospital for septic peritonitis. Of the nonsurvivors, 3 dogs died from cardiopulmonary arrest within 9 h of surgery. Four dogs were euthanized during surgery, 2 for nonresectable or metastatic neoplasia and 2 for extensive intestinal necrosis. Profound hypotension despite treatment with multiple vasopressors and the presence of moderate-to-severe peritonitis grossly were also recorded as factors contributing to intraoperative euthanasia in 2 dogs with neoplasia. Four dogs were euthanized during the postoperative period, 2 for multiple

© Veterinary Emergency and Critical Care Society 2013, doi: 10.1111/vec.12013

Table 1: Population characteristics of dogs with naturally occurring septic peritonitis

	Survivors ($n = 16$)	Nonsurvivors ($n = 11$)	*P*-value
Age (years)	6.5 ± 4.1	9.3 ± 2.8	0.06
Weight (kg)	24.8 ± 13.4	30.2 ± 17.4	0.4
Male	8 (50%)	5 (45%)	1.0
Female	8 (50%)	6 (55%)	
Neutered	12 (75%)	11 (100%)	0.1
Intact	4 males (25%)	0 (0%)	

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as the number of dogs (%) within each category.

organ failure, and 2 for persistent peritonitis requiring multiple laparotomies.

Population characteristics

Fifteen breeds of dogs were represented; mixed-breed dogs were the most common ($n = 8$). There were no statistically significant differences between survivors and nonsurvivors with respect to age, weight, proportion of males and females, or proportion of neutered and intact dogs (Table 1). All 27 dogs met criteria for SIRS; 26 dogs met criteria prior to surgery and 1 dog developed signs of SIRS postoperatively.

Surgical findings

The gastrointestinal tract was the most common source of septic peritonitis ($n = 18$), followed by the urogenital ($n = 5$) and hepatobiliary ($n = 2$) systems; 2 dogs had septic peritonitis due to another source (1 each of abscessed mesenteric lymph nodes and abscess of retained surgical sponge). Numerous etiologies were represented, but the most common were perforated gastrointestinal ulcer ($n = 5$), dehiscence of an enterotomy or intestinal anastomosis ($n = 5$), and perforating gastrointestinal foreign body ($n = 4$). Except for cases of intraoperative euthanasia, all dogs underwent primary closure of the peritoneal cavity, either with (20/23 [87%]) or without (3/23 [13%]) a closed-suction drain.

Samples of abdominal effusion were submitted for bacterial culture in 20 cases. Of the samples submitted for both aerobic and anaerobic culture ($n = 19$), 14 were positive only for aerobic bacteria, 2 were positive only for anaerobic bacteria, 2 were positive for both, and 1 culture was negative. The most commonly isolated organisms included *Escherichia coli* ($n = 10$) and *Enterococcus* spp. ($n = 7$).

Preoperative coagulation parameters and anticoagulant activities

The results of preoperative coagulation parameter testing and preoperative anticoagulant activity measurement are presented in Table 2. Mean AT activity was below the reference interval for both survivors and nonsurvivors; 14 of 15 (93%) survivors and 10 of 11 (91%) nonsurvivors had preoperative AT activity below the reference interval. Mean PC activity was below the reference interval for nonsurvivors; 10 of 11 (91%) nonsurvivors and only 2 of 15 (13%) survivors had preoperative PC activity below the reference interval.

Preoperative TEG

There was no difference in the *R*-value between survivors and nonsurvivors, although the median value for both groups was below the reference interval (Table 3). The *K* value was significantly lower (more hypercoagulable) among survivors, although the median value for both groups was below the reference interval. The remaining TEG values (α angle, MA, CI) were significantly greater (more hypercoagulable) among survivors compared to nonsurvivors, whose values were within the reference intervals.

Table 2: Preoperative (day 0) coagulation parameters and anticoagulant activities compared between survivors and nonsurvivors among dogs with naturally occurring septic peritonitis

	Units	Reference range	Survivors	*n*	Nonsurvivors	*n*	*P* value
Platelets	$\times 10^9$/L or $\times 10^3$ /μL	177–398	219 (182–251)	15	126 (68–227)	8	0.2
PT	% prolonged		0 (0–0)	14	0 (0–9)	11	0.05
aPTT	% prolonged		0 (0–0)	15	4 (0–31)	11	0.02
D-dimer	nmol/L	<1.1	3.40 ± 3.18	11	3.72 ± 2.96	8	0.8
	μg/mL	<0.2	0.62 ± 0.58		0.68 ± 0.54		
Fibrinogen	μmol/L	4.32–14.1	27.0 (23.9–31.1)	15	16.7 (8.85–18.7)	11	<0.001
	mg/dL	147–479	917 (813–1056)		567 (301–636)		
AT	%	65–145	53 ± 9	15	32 ± 16	11	<0.001
PC	%	75–135	98 ± 24	15	49 ± 26	11	<0.001

Continuous variables are expressed as mean ± SD or median (interquartile range).
PT, prothrombin time; aPTT, activated partial thromboplastin time; AT, antithrombin; PC, protein C.

© Veterinary Emergency and Critical Care Society 2013, doi: 10.1111/vec.12013

Table 3: Preoperative (day 0) thromboelastography parameters compared between survivors and nonsurvivors among dogs with naturally occurring septic peritonitis

	Reference range	Survivors ($n = 13$)	Nonsurvivors ($n = 6$)	*P* value
R (min)	5–7	3.8 (2.9–5.3)	4.3 (2.5–5.0)	0.9
K (min)	3–4	1.4 (1.3–1.8)	2.6 (2.0–3.2)	0.03
α angle (°)	44–56	68 ± 7	56 ± 10	<0.01
MA (mm)	54–61	71 ± 6	55 ± 10	<0.001
CI	−3 to 3	4.9 ± 1.1	2.9 ± 1.3	<0.01

Continuous variables are expressed as mean ± SD or median (interquartile range).
MA, maximum amplitude; CI, coagulation index.

Table 4: Receiver operator curve data predicting survival generated from preoperative (day 0) coagulation parameters, anticoagulant activities, and thromboelastography parameters among dogs with naturally occurring septic peritonitis

	Cut-off point	Sensitivity	Specificity	AUC	*P* value
PC	>60%	0.93	0.82	0.90	0.0006
AT	>41.5%	1.0	0.82	0.90	0.0006
Fibrinogen	>19.8 μmol/L >672 mg/dL	0.93	0.82	0.90	0.0006
K	<1.85 min	0.77	0.83	0.82	0.03
α angle	>65.8°	0.77	0.83	0.87	0.01
MA	>64.5 mm	0.85	1.0	0.95	0.002
CI	>2.05	0.85	0.67	0.80	0.04

AUC, area under curve; AT, antithrombin; PC, protein C; MA, maximum amplitude; CI, coagulation index.

Preoperative CBC and chemistry profile

There were no clinically relevant or statistically significant differences between survivors and nonsurvivors with respect to any of the preoperative CBC and biochemistry profile parameters.

ROC data

Preoperative routine coagulation parameters, including platelet count, percent prolongation of PT and aPTT, and D-dimer concentration did not provide predictive value based on ROCs. In contrast, PC and AT activities, fibrinogen concentration, and several TEG parameters (*K*, α angle, MA, and CI) were predictive of survival based on significant area under the curve on ROCs and cut-off values with high sensitivity and specificity (Table 4). The *R* value was the only preoperative TEG value that lacked predictive value based on ROC analysis.

ROC analysis of the data from day 1 is found in Table 5. The best predictors of survival were PC activity and the TEG parameters *K*, α angle, and MA. The CI was the only postoperative (day 1) TEG value that lacked predictive value based on ROC analysis. Postoperative (day 1) routine coagulation parameters, including platelet count, percent prolongation of PT and aPTT, and D-dimer concentration did not provide predictive value based on ROC analysis.

Table 5: Receiver operator curve data predicting survival generated from postoperative (day 1) coagulation parameters, anticoagulant activities, and thromboelastography parameters among dogs with naturally occurring septic peritonitis

	Cut-off point	Sensitivity	Specificity	AUC	*P* value
PC	>49%	0.93	1.0	0.97	0.005
AT	>22.5%	1.0	0.75	0.82	0.05
Fibrinogen	>18.3 μmol/L >622 mg/dL	0.67	1.0	0.83	0.05
R	<5.15 min	0.8	1.0	0.88	0.02
K	< 3.05 min	1.0	1.0	1.0	0.003
α angle	>52.4°	1.0	1.0	1.0	0.003
MA	>57 mm	1.0	1.0	1.0	0.003

AUC, area under curve; AT, antithrombin; PC, protein C; MA, maximum amplitude.

Serial data

Figures 1 and 2 display serial data for PC and AT activity, respectively, among both survivors and nonsurvivors. By day 3, only 2 of the 11 nonsurvivors were still alive, limiting the ability to analyze serial hemostatic changes. The proportion of survivors that showed an increase in PC activity between days 1 and 3 (8/11) was significantly greater than the proportion that showed an increase between days 0 and 1 (1/15) ($P < 0.001$). The day 3 PC values for survivors were not significantly different than the day 0 or 1 values; however, day 1 values were significantly lower than day 0 values ($n = 10$, $P < 0.05$). The proportion of survivors that showed an increase in AT activity between days 1 and 3 (11/11) was significantly greater than the proportion that showed an increase between days 0 and 1 (2/15) ($P < 0.001$). Day 1 AT activity among survivors was significantly lower than values for both days 0 and 3; by day 3 AT activity was significantly higher than day 0 ($P < 0.05$). None of the TEG values (*R*, *K*, α angle, MA, or CI), however, were significantly different over time among the survivors ($n = 13$ on day 0, $n = 16$ on day 1, and $n = 10$ on day 3).

Discussion

Results of this study of dogs with naturally occurring septic peritonitis highlight the complexity of the hemostatic imbalance in canine sepsis by documenting elements of both hypercoagulability and hypocoagulability. Several results suggest that the overall hemostatic profile of nonsurvivors may have been most consistent with a consumptive, decompensated state. Similar to the findings by deLaforcade et al,[2,3] PC and AT deficiency

© Veterinary Emergency and Critical Care Society 2013, doi: 10.1111/vec.12013

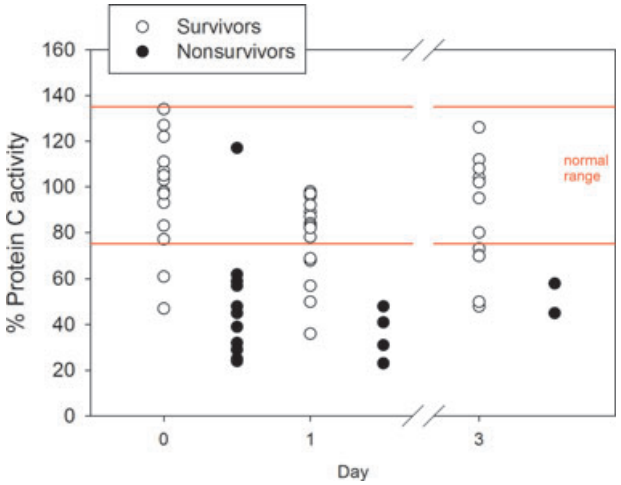

Figure 1: Serial data for protein C activity among dogs with naturally occurring septic peritonitis. Dot plot of protein C activity in plasma of survivors (open circles) and nonsurvivors (closed circles) prior to surgery for septic peritonitis (day 0; $n = 15$ survivors, $n = 11$ nonsurvivors), the day after surgery (day 1; $n = 16$ survivors, $n = 4$ nonsurvivors), and 3 days after surgery (day 3; $n = 11$ survivors, $n = 2$ nonsurvivors).

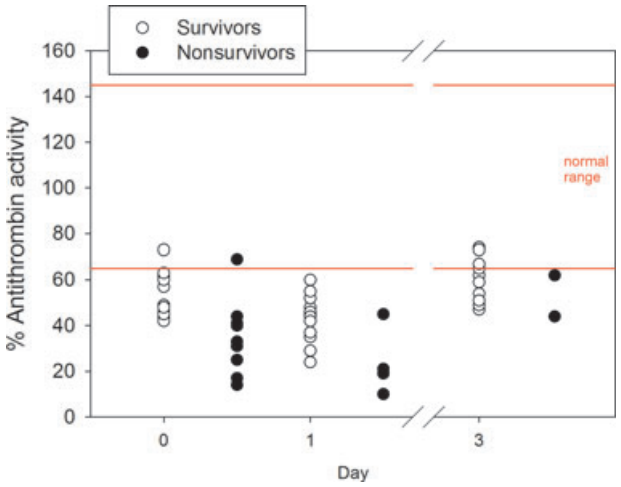

Figure 2: Serial data for antithrombin activity among dogs with naturally occurring septic peritonitis. Dot plot of antithrombin activity in plasma of survivors (open circles) and nonsurvivors (closed circles) prior to surgery for septic peritonitis (day 0; $n = 15$ survivors, $n = 11$ nonsurvivors), the day after surgery (day 1; $n = 16$ survivors, $n = 4$ nonsurvivors), and 3 days after surgery (day 3; $n = 11$ survivors, $n = 2$ nonsurvivors).

characterized the hemostatic imbalance of the dogs in this study, although the anticoagulant deficiencies were more severe among the nonsurvivors. The hemostatic profile of the nonsurvivors was also characterized by loss of procoagulant factors as demonstrated by prolongation of aPTT, although the PT and platelet count were similar to survivors. The less hypercoagulable thromboelastograms among nonsurvivors may reflect a status of relative hypocoagulability compared to survivors as has been documented in other patient populations.[25–27] In contrast, survivors had hypercoagulable thromboelastograph parameters, were able to maintain activity of PC, and had less severe depletion of AT activity, suggesting a compensated, procoagulant state. Nonsurvivors either failed to generate a compensated, hypercoagulable state or had progressed to a generalized consumption of both pro- and anticoagulant factors. The increased D-dimer concentration among both survivors and nonsurvivors may support the latter interpretation that excessive thrombosis was a component of the hemostatic imbalance among nonsurvivors at some point in time.

The results of this study confirmed the prognostic significance of PC and AT deficiency in naturally occurring canine sepsis. Unlike previous studies of naturally occurring sepsis in a more heterogeneous population of dogs,[2,3] results of this study documented a significant difference among survivors and nonsurvivors in both PC and AT activities at the first sampling point. These results and the results of the ROC analysis demonstrate that PC and AT deficiencies early in septic peritonitis, as well as postoperatively, were predictive of mortality. The inconsistency in identifying anticoagulant activities as an early indicator of outcome in dogs in previous studies may arise from differences in sample size and patient heterogeneity.

PC and AT activities showed a similar pattern over time among survivors, decreasing from days 0 to 1. The physiologic challenges that anesthesia and surgery present for these critically ill patients may be partly responsible for the decline. In a previous study, deLaforcade et al[3] documented a similar trend in anticoagulant activities among a group of surgical and nonsurgical septic dogs. In people, absolute PC activity, rather than change in PC activity, was correlated with mortality, whereas both the absolute value and change in activity of AT were associated with mortality.[28] Although there were insufficient data to analyze a trend for nonsurvivors over time, day 3 data for survivors show recovery of anticoagulant activities. A similar trend with recovery of anticoagulant activities among survivors and either lack of recovery or progressive decline of anticoagulant activities among nonsurvivors has been documented in septic people.[12,13]

Although the mechanisms for the protective effects of PC and AT were not investigated in this study, several possible mechanisms exist. Severe PC and AT deficiency is associated with the loss of both anticoagulant and anti-inflammatory activity.[29] PC also has cytoprotective effects, including maintaining the viability of lymphocytes and endothelial cells, and profibrinolytic effects.[30] While the anticoagulant and profibrinolytic effects of PC are

mediated by direct enzyme-substrate interactions with factors Va and VIIa and plasminogen activator inhibitor-1, respectively, the anti-inflammatory and cytoprotective effects of PC are mediated by 2 receptors (endothelial cell PC receptor and protease-activated receptor 1).[30] Kerschen et al[31] examined the relative importance of the activities of PC in an experimental model of murine sepsis by treating mice with either wild-type activated PC or a recombinant variant of activated PC with normal receptor signaling but reduced anticoagulant activity. The recombinant variant of activated PC increased survival among mice with peritonitis, suggesting that the anti-inflammatory and cytoprotective activities of PC are important mechanisms for its protective effects in sepsis. The results of other experimental studies of murine peritonitis suggest that loss of the fibrinolytic activity of PC promotes bacterial growth by allowing bacteria to remain sequestered within intra-abdominal fibrin.[32,33]

Preoperative fibrinogen concentration had strong predictive value for outcome among dogs in this study. Evaluation of fibrinogen concentration in septic people has yielded variable results, limiting its prognostic value.[12,34,35] The variability of fibrinogen concentration may be due to the combined roles of fibrinogen in both hemostatic balance and as an acute phase inflammatory protein. Fibrinogen concentration was correlated with C reactive protein in one population of septic people, suggesting that the increase in fibrinogen concentration was secondary to an acute phase inflammatory reaction.[34] The fibrinogen concentration among both survivors and nonsurvivors in the present study may have been increased at least in part due to an acute inflammatory response. The increased fibrinogen concentration may have contributed to the hypercoagulability seen particularly among the survivors in this study, as has been documented in septic people.[36]

TEG has been used to characterize hemostatic dysfunction in dogs with a variety of diseases, but TEG results have not been previously reported in dogs with septic peritonitis.[18,19,25–27,37–39] Overall, the dogs in this study had thromboelastograms that were either normal or hypercoagulable. Unlike PC and AT, however, TEG values did not trend back toward normal by day 3 among survivors, suggesting the complexity of the hypercoagulable state. Hypercoagulability has also been documented in septic people.[36] In one population of septic people, TEG documented a delay in clot initiation but an enhanced rate of clot formation and increased clot strength once clot initiation had begun. Patients in that study, such as the dogs in this study, had variable levels of procoagulant and anticoagulant proteins, which highlights the potential usefulness of TEG in providing a global interpretation of multiple and often mixed alterations of the individual components of coagulation.[34]

TEG was sensitive and specific in distinguishing survivors and nonsurvivors in the population of dogs in this study. The *K*, α angle, and MA parameters had strong predictive value, particularly on day 1 postoperatively, each achieving a sensitivity and specificity of 100%. In addition to its potential use as a prognostic indicator, TEG may allow earlier diagnosis and treatment of hemostatic imbalance, particularly hypercoagulability, than routine coagulation tests.[18] In conjunction with assays of specific markers of inflammation, TEG may be useful in future studies to determine the relative significance of the anticoagulant and anti-inflammatory effects of PC and AT and the fibrinolytic effects of PC.

In addition, the interpretation of the hypercoagulable state may have been influenced by the TEG methodology, which did not provide a source of contact activation.[40] This study was performed using recalcified citrated blood with no activating reagent. These conditions require the generation of active factor XII through the contact pathway to support assembly of coagulation complexes that ultimately generate thrombin.[40] The lack of a trigger reagent increases the assay sensitivity to ex vivo variability in contact pathway activation, which has most pronounced effects on the time-lag until initial fibrin formation.[40]

Routine coagulation tests, including platelet count, PT, aPTT, and D-dimer concentration, were not useful in distinguishing survivors and nonsurvivors. Although there was a difference in aPTT between the two groups, the difference may not be substantial enough to render aPTT useful as a prognostic indicator clinically. Evaluation of the prognostic value of routine coagulation tests in septic people has yielded inconsistent results. Both PT and D-dimer concentration were predictive of organ failure and mortality in one population of septic people, although a composite coagulopathy score including several variables was of greater predictive value.[28] Other studies have failed to support the predictive value of D-dimer concentration, aPTT, and platelet count.[13,14,28]

In this study, the tests of coagulation that had the greatest predictive value are not as widely available on a clinical basis as the routine coagulation tests. Among these specialized tests, fibrinogen concentration is the most readily available since it is offered by large private and university diagnostic laboratories and is accessible as a POC analyzer.[41] TEG is only available at hospitals with a TEG analyzer on site because the blood sample must be processed within 30 min of venipuncture, and assays for anticoagulant activities in dogs are not yet widely available on a clinical basis.[19] However, studies such as this one that demonstrate the clinical value of TEG, PC, and AT may promote the availability of these tests, at least at tertiary care centers, or the development of POC tests.

© Veterinary Emergency and Critical Care Society 2013, doi: 10.1111/vec.12013

An assay for the activated form of PC is not available for dogs. Although diminished activation contributes to PC deficiency in sepsis, PC deficiency can also result from consumption, degradation, and inadequate production.[42] Thus, we expect that documenting PC deficiency with the assay used in this study represents a clinically relevant imbalance in hemostasis.

Methods for evaluating disease severity are not standardized in veterinary medicine. SIRS criteria are of low specificity and are of limited clinical value.[22,43] However, the overall survival rate of 59% in this study is consistent with reported survival rates, suggesting that our population is representative of dogs with septic peritonitis.[4–11]

There are several limitations to this study. The relatively small sample size of the study limits its statistical power, particularly for serial data. The particular difficulty in obtaining serial data from nonsurvivors is not surprising given that death due to septic peritonitis often occurs in the early postoperative period in dogs.[5,7,9] In addition, data were unavailable in some cases for both survivors and nonsurvivors due to limitations in the availability of clinical trial staff and laboratory equipment. Applying a statistical correction for multiple comparisons reduces type I error but increases type II error and may not be recommended in all studies in which multiple comparisons are performed.[23,24] Even if such a correction had been applied to the data in this study, the strongest results would remain statistically significant. As is the case for many veterinary studies, the interpretation of survival as an outcome may be complicated by the fact that both euthanasia and natural death were outcomes among the nonsurvivors. Although all dogs in this study underwent surgical treatment of septic peritonitis, heterogeneity still exists within the population itself, and there was variability in patient management, which was at the discretion of the primary clinician. Finally, it is likely that there were additional factors affecting the prognosis of the study population that were neither identified nor controlled.

Despite these limitations, the results of this study provide clear evidence of hemostatic imbalance in dogs with septic peritonitis. These findings are consistent with hemostatic imbalance in human septic patients.[12–16,28,34] As a result, additional clinical studies in dogs with naturally occurring septic peritonitis may provide a spontaneous model for investigating novel treatments in human sepsis, which will also benefit the treatment of dogs. Additional studies of naturally occurring sepsis in dogs are also needed to evaluate the inclusion of the coagulation parameters examined in this study into composite scores to measure disease severity and determine prognosis, as has been done for septic people.[28,44]

Acknowledgments

The authors thank Dr. Marjory Brooks from the Comparative Coagulation Section, Animal Health Diagnostic Laboratory of Cornell University, for determination of anticoagulant activities and fibrinogen concentration and Dr. James Baumgardner, from Oscillogy LLC, Folsom, PA, for statistical advice.

Footnotes

[a] Vitros 250 chemistry analyzer, Ortho-Clinical Diagnostics, Johnson & Johnson, New Brunswick, NJ.
[b] Cell-Dyn 3200, Abbott Diagnostics, Abbott Park, IL.
[c] STA-Compact, Diagnostica Stago, Parsippany, NJ.
[d] STA neoplastine rabbit brain thromboplastin with heparin inhibitor (PT); STA CK PREST 5 cephalin reagent with kaolin activator (aPTT); STA LIATEST D-DI (D-dimer), Diagnostica Stago.
[e] SCA-2000 (PT and aPTT), Synbiotics, San Diego, CA.
[f] TEG Hemostasis Analyzer, Haemoscope Corporation, Niles, IL.
[g] Stachrom AT III and Stachrom Protein C, Diagnostica Stago.
[h] Fibrinogen, Diagnostica Stago.
[i] SigmaPlot 11, Systat Software, Evanston, IL.

References

1. Hardie EM, Rawlings CA, Calvert CA. Severe sepsis in selected small animal surgical patients. J Am Anim Hosp Assoc 1986; 22:33–41.
2. deLaforcade AM, Freeman LM, Shaw SP, et al. Hemostatic changes in dogs with naturally occurring sepsis. J Vet Intern Med 2003; 17:674–679.
3. deLaforcade AM, Rozanski EA, Freeman LM, et al. Serial evaluation of protein C and antithrombin in dogs with sepsis. J Vet Intern Med 2008; 22:26–30.
4. Ludwig LL, McLoughlin MA, Graves TK, et al. Surgical treatment of bile peritonitis in 24 dogs and 2 cats: a retrospective study (1987–1994). Vet Surg 1997; 26:90–98.
5. King LG. Postoperative complications and prognostic indicators in dogs and cats with septic peritonitis: 23 cases (1989–1992). J Am Vet Med Assoc 1994; 204:407–414.
6. Staatz AJ, Monnet E, Seim HB. Open peritoneal drainage versus primary closure for the treatment of septic peritonitis in dogs and cats: 42 cases (1993–1999). Vet Surg 2002; 31:174–180.
7. Mueller MG, Ludwig LL, Barton LJ. Use of closed-suction drains to treat generalized peritonitis in dogs and cats: 40 cases (1997–1999). J Am Vet Med Assoc 2001; 219:789–794.
8. Lanz OI, Ellison GW, Bellah JR, et al. Surgical treatment of septic peritonitis without abdominal drainage in 28 dogs. J Am Anim Hosp Assoc 2001; 37:87–92.
9. Greenfield CL, Walshaw R. Open peritoneal drainage for treatment of contaminated peritoneal cavity and septic peritonitis in dogs and cats: 24 cases (1980–1986). J Am Vet Med Assoc 1987; 191:100–105.
10. Woolfson JM, Dulisch ML. Open abdominal drainage in the treatment of generalized peritonitis in 25 dogs and cats. Vet Surg 1986; 15:27–32.
11. Bentley AM, Otto CM, Shofer FS. Comparison of dogs with septic peritonitis: 1988–1993 versus 1999–2003. J Vet Emerg Crit Care 2007; 17:391–398.
12. Lorente JA, Garcia-Frade LJ, Landin L, et al. Time course of hemostatic abnormalities in sepsis and its relation to outcome. Chest 1993; 103:1536–1542.
13. Karamarkovic A, Radenkovic D, Milic N, et al. Protein C as an early marker of severe septic complications in diffuse secondary peritonitis. World J Surg 2005; 29:759–765.
14. Fisher CJ, Yan SB. Protein C levels as a prognostic indicator of outcome in sepsis and related diseases. Crit Care Med 2000; 28:S49–S56.

15. Vangerow B, Shorr AF, Wyncoll D, et al. The protein **C** pathway: implications for the design of the RESPOND study. Crit Care 2007; 11(Suppl 5):S4.
16. Macias WL, Nelson DR. Severe protein C deficiency predicts early death in severe sepsis. Crit Care Med 2004; 32:S223–S228.
17. Donahue SM, Otto CM. Thromboelastography: a tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. J Vet Emerg Crit Care 2005; 15:9–16.
18. Donahue SM, Brooks M, Otto CM. Examination of hemostatic parameters to detect hypercoagulability in dogs with severe protein-losing nephropathy. J Vet Emerg Crit Care 2011; 21:346–355.
19. Otto CM, Rieser TM, Brooks MB, et al. Evidence of hypercoagulability in dogs with parvoviral enteritis. J Am Vet Med Assoc 2000; 217:1500–1504.
20. Cohen E, Caprini J, Zuckerman L, et al. Evaluation of three methods used to identify accelerated coagulability. Thromb Res 1977; 10:577–604.
21. Caprini J, Zuckerman L, Cohen E, et al. The identification of accelerated coagulability. Thromb Res 1976; 9:167–180.
22. Hauptman JG, Walshaw R, Olivier NB. Evaluation of the sensitivity and specificity of diagnostic criteria for sepsis in dogs. Vet Surg 1997; 26:393–397.
23. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology 1990; 1:43–46.
24. Streiner DL, Norman GR. Correction for multiple testing: is there a resolution? Chest 2011; 140:16–18.
25. Sinnott VB, Otto CM. Use of thromboelastography in dogs with immune-mediated hemolytic anemia: 39 cases (2000–2008). J Vet Emerg Crit Care 2009; 19:484–488.
26. Wiinberg B, Jensen AL, Johansson PI, et al. Thromboelastographic evaluation of hemostatic function in dogs with disseminated intravascular coagulation. J Vet Intern Med 2008; 22:357–365.
27. Goggs R, Wiinberg B, Kjelgaard-Hansen, et al. Serial assessment of the coagulation status of dogs with immune-mediated haemolytic anaemia using thromboelastography. Vet J 2012; 191(3):347–353.
28. Dhainaut JF, Shorr AF, Macias WL, et al. Dynamic evolution of coagulopathy in the first day of severe sepsis: relationship with mortality and organ failure. Crit Care Med 2005; 33:341–348.
29. Hopper K, Bateman S. An updated view of hemostasis: mechanisms of hemostatic dysfunction associated with sepsis. J Vet Emerg Crit Care 2005; 15:83–91.
30. Esmon CT. Inflammation and the activated PC anticoagulant pathway. Semin Thromb Hemost 2006; 32(Suppl 1):49–60.
31. Kerschen EJ, Fernandez JA, Cooley BC, et al. Endotoxemia and sepsis mortality reduction by non-anticoagulant-activated protein C. J Exp Med 2007; 204:2439–2448.
32. van Veen SQ, Levi M, van Vilet AK, et al. Peritoneal lavage with activated protein C alters compartmentalized coagulation and fibrinolysis and improves survival in polymicrobial peritonitis. Crit Care Med 2006; 34:2799–2805.
33. Schouten M, van't Veer C, Levi M, et al. Endogenous protein C inhibits activation of coagulation and transiently lowers bacterial outgrowth in murine ***E****scherichia coli* peritonitis. J Thromb Haemost 2011; 9:1072–1075.
34. Collins PW, Macchiavello LI, Lewis SJ, et al. Global tests of haemostasis in critically ill patients with severe sepsis syndrome compared to controls. Brit J Haematol 2006; 135:220–227.
35. Yan SB, Helterbrand JD, Hartman DL, et al. Low levels of protein C are associated with poor outcome in severe sepsis. Chest 2001; 120:915–922.
36. Gonano C, Sitzwohl C, Meitner E, et al. Four-day AT therapy does not seem to attenuate hypercoagulability in patients suffering from sepsis. Crit Care 2006; 10:R160.
37. Kristensen AT, Wiinberg B, Jessen LR, et al. Evaluation of human recombinant tissue factor-activated thromboelastography in 49 dogs with neoplasia. J Vet Intern Med 2008; 22:140–147.
38. Fenty RK, de Laforcade AM, Shaw SP, et al. Identification of hypercoagulability in dogs with primary immune-mediated hemolytic anemia by means of thromboelastography. J Am Vet Med Assoc 2011; 238:463–467.
39. Goodwin LV, Goggs R, Chan DL, et al. Hypercoagulability in dogs with protein-losing enteropathy. J Vet Intern Med 2011; 25: 273–277.
40. Smith AS, McMichael M, Galligan A, et al. Clot formation in canine whole blood as measured by rotational thromboelastometry is influenced by sample handling and coagulation activator. Blood Coagul Fibrinolysis 2010; 21:692–702.
41. Mineo HK, Garabed RB. Evaluation of a bench-top coagulation analyzer for measurement of prothrombin time, activated partial thromboplastin time, and fibrinogen concentrations in healthy dogs. Am J Vet Res 2007; 68:1342–1347.
42. Levi M, van der Poll T. Recombinant human activated protein C: current insights into its mechanism of action. Crit Care 2007; 11(Suppl 5):S3.
43. Howell, MD, Talmor D, Schuetz P, et al. Proof of principle: the predisposition, infection, response, organ failure sepsis staging system. Crit Care 2011; 39:322–327.
44. Shapiro NI, Trzeciak S, Hollander JE, et al. A prospective, multicenter derivation of a biomarker panel to assess risk of organ dysfunction, shock, and death in emergency department patients with suspected sepsis. Crit Care Med 2009; 37:96–104.

© Veterinary Emergency and Critical Care Society 2013, doi: 10.1111/vec.12013