

Evaluation of coagulation in dogs with partial or complete extrahepatic biliary tract obstruction by means of thromboelastography

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Objective—To characterize in vitro coagulation status in a cohort of dogs with extrahepatic biliary tract obstruction (EHBO) and to evaluate these patients for hypercoagulability by means of thromboelastography.

Design—Prospective cohort study.

Animals—10 dogs with EHBO and 19 healthy control dogs.

Procedures—Partial or complete EHBO was confirmed via exploratory celiotomy. Venous blood samples were collected for evaluation of prothrombin time (PT) and activated partial thromboplastin time (APTT); fibrinogen and D-dimer concentrations; protein C and antithrombin activities; and factor VII, VIII, and XI coagulant activities in plasma as well as thromboelastography in whole blood. Thromboelastography variables were measured from the thromboelastography tracing, and a coagulation index was calculated. Thromboelastography results were compared with those of healthy control dogs previously evaluated by the same laboratory.

Results—Hypercoagulability was diagnosed in all dogs with EHBO on the basis of a high coagulation index. Thromboelastography variables, including maximal amplitude, α -angle, and coagulation index, were significantly higher, and K (clot formation time) and R (reaction time) were significantly lower in these dogs than in control dogs. All dogs with EHBO had PT and APTT within respective reference ranges. Plasma D-dimer and fibrinogen concentrations were above reference ranges in 8 and 7 dogs, respectively, and protein C and antithrombin activities were below reference ranges in 3 and 1 dogs, respectively.

Conclusions and Clinical Relevance—In vitro hypercoagulability was commonly detected in dogs with naturally occurring EHBO. The traditional view of EHBO as a disease that causes hypocoagulability may need to be reconsidered. (*J Am Vet Med Assoc* 2013;242:778–785)

Extrahepatic biliary tract obstruction in dogs can have many potential etiologies, including pancreatitis, neoplasia, cholangiohepatitis, and cholelithiasis.^{1–3} Management of these patients is frequently a major challenge for veterinarians, regardless of underlying cause. Not only do they often require technically complex surgery to provide relief of obstruction, but they may also have substantial systemic compromise.^{1–3} The complete obstruction of bile flow into the duodenum can have physiologic consequences on numerous body systems. Alterations in normal coagulation pathways are among the most clinically relevant factors in patients that may require surgery on an emergency basis.

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ABBREVIATIONS

APTT	Activated partial thromboplastin time
EHBO	Extrahepatic biliary tract obstruction
MA	Maximal amplitude
PT	Prothrombin time

Coagulation factors II, VII, IX, and X and the anticoagulant factors protein C and protein S all require vitamin K–dependent carboxylation of glutamic acid residues to become hemostatically active.⁴ Patients with EHBO theoretically have a decreased concentration or complete lack of bile salts in the small intestine, resulting in an inability to emulsify dietary fats. These fats are essential for intestinal absorption of fat-soluble vitamin K, with their deficiency leading to impaired absorption of vitamin K in this patient population and, potentially, defective posttranslational processing of vitamin K–dependent factors.⁵ The risk for vitamin K deficiency has led to the general assumption that EHBO is a disorder that causes hypocoagulability. Other factors, including endotoxemia-induced activation of coagulation; relative activities of the physiologic anticoagulants antithrombin, protein C, and protein S; and the presence of concurrent hepatic disease or disseminated

intravascular coagulation, may play a role in determining whether procoagulant or anticoagulant imbalance develops in patients with EHBO. Recently, there has been a paradigm shift from the classic cascade model of coagulation, which consists of sequential cleavage of zymogen substrates on phospholipid membrane surfaces in the presence of calcium resulting in formation of fibrin, to the cell-based model of coagulation. The latter incorporates the roles of cells expressing tissue factor in initiating coagulation *in vivo* and activated platelets in providing a procoagulant membrane surface for the accelerated generation of factor Xa and factor IIa.⁶ The cell-based model of coagulation is superior to the cascade model for explaining hypercoagulability associated with increased platelet reactivity following major surgical trauma.⁷

Both hypocoagulability and hypercoagulability have been described in human patients with EHBO.^{8–11} There is comparatively little information in the veterinary literature on the spectrum of coagulation disorders in dogs and cats with naturally occurring EHBO. Thromboelastography is a diagnostic tool for studying complex coagulopathies. The technique is used to evaluate dynamic changes in clot strength from the initiation of fibrin formation through maturation and fibrinolysis.¹² Thromboelastography has been used to characterize hypo- and hypercoagulable states in people, and a correlation between thromboelastography results and clinical signs of bleeding, but not thrombosis, has been reported in dogs.¹³ A better understanding of hemostatic balance in dogs with EHBO may lead to an improvement in the currently high rates of perioperative morbidity and mortality in this patient population.^{1–3} Our hypothesis was that a hypercoagulable state is present in some dogs with naturally occurring EHBO, similar to human patients with the disease. The aims of the study reported here were to characterize the *in vitro* coagulation status of dogs with naturally occurring EHBO and to screen for evidence of hypercoagulability on the basis of thromboelastography results.

Materials and Methods

Dogs—Ten dogs with naturally occurring partial or complete EHBO were prospectively enrolled in the study over a 2-year period between September 1, 2006, and September 9, 2008. Owner consent was obtained for all dogs enrolled in the study. The protocol was approved by the Institutional Animal Care and Use Committee.

Criteria for inclusion were as follows: confirmation of partial or complete EHBO by means of exploratory celiotomy and having a complete medical record to the time of euthanasia or discharge from the hospital. Dogs were categorized as having complete EHBO if bile could not be expressed from the extrahepatic biliary tree into the duodenum at surgery or necropsy and as having partial EHBO if results of clinicopathologic analysis and diagnostic imaging were consistent with extrahepatic cholestasis and, at surgery, a subjectively increased resistance to expression of bile from the biliary tree into the duodenum was present, despite some bile passing into the duodenum when the gallbladder was expressed. Dogs were excluded from the study if they had received vitamin K supplementation or had

been administered blood products prior to sample collection or if they were receiving any medication (including NSAIDs, corticosteroids, heparin, or hydroxyethyl starch) known to influence platelet function, hemostatic proteins, or fibrin clot formation. Dogs were also excluded if they had clinical or clinicopathologic evidence of concurrent diseases known to be associated with a hypercoagulable state, such as hyperadrenocorticism, parvoviral enteritis, immune-mediated hemolytic anemia, and protein-losing nephropathy. All patients were treated according to the standard of care for management of dogs with EHBO, and all case management decisions, including additional diagnostic testing, were at the discretion of the supervising clinician.

Laboratory data from 19 healthy dogs that had undergone thromboelastography evaluation and had antithrombin and fibrinogen assays performed in the same clinical laboratories as dogs with EHBO were available for statistical comparison. Some data from this control population were previously published.^{14,15}

Data collection—Data recorded included patient sex, breed, age, surgical findings, underlying cause of EHBO, presence of partial or complete EHBO, presence or absence of bile peritonitis, results of microbial culture and susceptibility testing, and histologic findings for tissue samples from the liver, gallbladder, or bile collected during surgery. Outcome (euthanasia or discharge from the hospital) was also recorded.

Blood samples—All blood samples used in evaluation of coagulation were collected ≤ 24 hours prior to surgery, once a high index of suspicion for EHBO existed and owners had consented to surgical exploration. Approximately 6 mL of blood was obtained from each dog with minimal trauma via cephalic or jugular venipuncture. Blood was transferred into 2 evacuated tubes containing one-tenth the final volume of 3.8% sodium citrate as the anticoagulant for thromboelastography and other coagulation testing. Samples for thromboelastography analysis were stored at room temperature (approx 22°C) and analyzed ≤ 30 minutes after blood collection. Blood samples for other coagulation tests were placed on ice and centrifuged, with citrated plasma removed and assayed immediately (PT and APTT) or frozen (-20° to -70° C) and subsequently shipped on ice within 3 days after sample collection to the Cornell University Comparative Coagulation Laboratory. Blood samples for serum biochemical analysis and CBCs were placed into serum tubes and EDTA-anticoagulant tubes, respectively.

CBC and serum biochemical analysis—The CBCs were performed by use of an automated cell counter,^a with a manual review of blood smears by a laboratory technician to verify platelet counts. Serum biochemical analysis was performed with an automated analyzer^b; concentrations of electrolytes, BUN, creatinine, total protein, albumin, globulin, total bilirubin, and cholesterol and activities of serum alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase were assessed.

Coagulation assays—All in-house coagulation tests were performed at 1 time point ≤ 24 hours prior

to surgery. Prothrombin time and APTT assays, D-dimer concentration determination, and thromboelastography were performed at the clinical laboratory of the Ryan Veterinary Hospital of the University of Pennsylvania. Prothrombin time and APTT were determined by use of an automated coagulation instrument^c with a mechanical endpoint detection method. D-dimer concentration was measured with a commercially available latex-agglutination kit.^d Thromboelastography was performed with a computerized thromboelastography system^e as described.¹⁰ Samples were recalcified, but no additional activators were used to accelerate clotting. Thromboelastography variables recorded from the tracings included R (reaction time), expressed in minutes, representing time from activation of the blood sample with calcium until the arms of the thromboelastography tracing are 2 mm apart; α -angle, expressed in degrees, representing the angle created by the tangent to the thromboelastography tracing at a width of 2 mm; K (clot formation time), expressed in minutes, representing the time it takes the 2 arms of the tracing to diverge by 20 mm; and MA, expressed in millimeters, representing the maximum width between the 2 arms of the thromboelastography tracing or the overall clot strength. From these 4 variables, a coagulation index was calculated via a previously derived formula and compared with a reference range calculated for control dogs tested by the same laboratory.^{12,14–16} Dogs with a coagulation index above the reference range were defined as having hypercoagulability on the basis of published reference values.^{12,14–16}

Plasma fibrinogen concentration, antithrombin activity, protein C activity, factor VII coagulant activity (in 5/10 dogs), factor VIII coagulant activity (in 5/10 dogs), and factor XI coagulant activity were assayed at the Comparative Coagulation Laboratory at Cornell University. Fibrinogen was measured via the Clauss method, as described¹⁷; because of a clerical error, factor VIII coagulant activity was inadvertently assayed in 5 dogs instead of factor VII coagulant activity. Plasma antithrombin and protein C activities were measured with commercial chromogenic kits^{f,g} and the manufacturer's coagulation instrument.^h These assays were modified by the use of a pooled canine plasma sample (prepared from 15 healthy dogs) as the assay calibration standard. Factor activities were measured in 1-stage modified PT (factor VII coagulant activity) or APTT (factor VIII coagulant activity and factor XI coagulant activity) assays, with canine or bovine substrate-deficient plasmas, as described.¹⁸ The results for antithrombin and protein C activities and factor VII, VIII, and XI coagulant activities were reported as the percentage activity of the canine plasma standard, which had an assigned value of 100% activity.

Statistical analysis—Descriptive statistics were calculated. Continuous data were expressed as means and SD, unless not normally distributed, in which case median values and ranges were reported. Categorical data were expressed as frequencies. To assess the comparability of the EHBO and control groups, a Fisher exact test was used to evaluate differences in control versus EHBO groups with regard to sex (male vs female) and breed category (mixed breed vs purebred) and,

because the data were normally distributed, Student *t* tests were used to determine whether there were significant differences in age and body weight between the control and EHBO groups. In addition, Student *t* tests were used to evaluate differences in MA, α -angle, coagulation index, antithrombin activity, and fibrinogen concentration between the control and EHBO groups. Because the values were not normally distributed, a Mann-Whitney test was used to determine whether there were significant differences in R and K between the control and EHBO groups. All analyses were 2 tailed, and significance was set at $P < 0.05$. To control for any differences that existed between groups (control vs EHBO) in age, body weight, sex, or breed, multiple linear regression analysis was performed to control for these factors and evaluate the association of EHBO versus control group on the various coagulation variables. In addition, multiple linear regression analysis was performed to evaluate associations of total bilirubin concentration, fibrinogen concentration, protein C activity, and Hct with MA and coagulation index. For regression modeling, 2-way interactions among the main effects were investigated. Univariate analysis was performed, and variables with a value of $P < 0.20$ were evaluated in a multivariable model. A given variable was retained in the multivariable model when the value of P for that variable was ≤ 0.05 . Model assumptions of linearity, equality of variance (homoscedasticity), and error term normality were assessed with scatterplots and histograms. In addition, evidence for heteroscedasticity and nonnormal skewness was evaluated with Cameron and Trivedi decomposition of the information matrix test. Because of the generally small sample size, linear regression analyses were repeated for those models in which there were outliers for the coagulation variables, to inform as to how robust the results might be. All analyses were performed with commercially available statistical software.¹

Results

Animals—Ten dogs met the inclusion criteria for the EHBO group, and data from 19 previously identified healthy control dogs were used for comparison of results of thromboelastography and antithrombin and fibrinogen assays. Mean \pm SD weight of dogs in the EHBO and control groups was 19.7 ± 14.4 kg (43.3 ± 31.7 lb) and 23.2 ± 8.4 kg (51.0 ± 18.5 lb), respectively. Mean \pm SD age of dogs in the EHBO and control groups was 9.6 ± 2.3 years and 5.4 ± 3.6 years, respectively ($P = 0.03$). Of 10 dogs with EHBO, 2 were Golden Retrievers and the remaining dogs were of 8 other breeds. Overall, 9 dogs with EHBO were purebred and 1 was mixed breed, whereas only 8 control dogs were purebred, and the remaining 11 were mixed breed ($P = 0.019$). In the EHBO group, 7 dogs were spayed females, 1 was a sexually intact female, and 2 were castrated males. In the control group, 13 dogs were spayed females and 6 were castrated males.

CBC, serum biochemical analysis, and coagulation assay results—Blood samples for coagulation assays were collected a median of 220 minutes (range, 75 to 1,260 minutes) prior to surgery in 8 of 10 dogs

with EHBO. In the remaining 2 dogs, the exact time of surgery was not recorded, but samples were collected prior to and on the same day as surgery (ie, < 24 hours before surgery). Results of CBC and serum biochemical analysis were summarized; complete serum biochemical analysis was performed for 9 of 10 dogs. (Table 1). Coagulation variables were reported (Table 2). All 10 dogs had PT and APTT values within the laboratory reference range; plasma fibrinogen and D-dimer concentrations were high in 7 and 8 dogs, respectively. Antithrombin activity was below the reference range in only 1 dog, whereas protein C activity was low in 3 dogs and slightly above the reference range in 1. One of the 5 dogs that underwent factor VII coagulant activity testing had a slightly low value, and 2 of 5 dogs had concomitant high values for factor VIII and factor XI coagulant activities.

Results of thromboelastography analysis revealed that R was below the reference range in 9 of 10 dogs with EHBO, and all 10 dogs had a decreased K and an increased α -angle, compared with reference values. Maximal amplitude was increased in 8 dogs, and the calculated coagulation index was high in all dogs.

These thromboelastography values were consistent with a hypercoagulable state^{12,14-16} in all 10 dogs in the EHBO group.

Significant differences were detected in plasma fibrinogen concentration, α -angle, MA, R, K, and coagulation index between the control and EHBO groups (Table 2). Three control dogs had outlier values for K (6.4, 7.0, and 7.9 minutes), and 2 control dogs had an outlier value for R (10.2 and 13.6 minutes). In linear regression analysis, neither age nor breed was found to be a confounder for the association of EHBO with fibrinogen concentration, R, or coagulation index (ie, the coefficient for EHBO did not change by > 15% when age or breed was added to the model). Dogs with EHBO had a mean fibrinogen concentration 551 mg/dL greater (95% CI, 395 to 706; $P < 0.001$), median R 3.0 minutes less (95% CI, 4.8 to 1.4; $P = 0.001$) with outliers included or 2.9 minutes less (95% CI, 4.6 to 1.2; $P = 0.001$) with outliers excluded, and mean coagulation index 3.6 units greater (95% CI, 2.6 to 4.6; $P < 0.001$) than did control dogs. Age was found to be a confounder in the association between EHBO and K, α -angle, and MA. Controlling for age, dogs with

Table 1—Selected results of CBC and serum biochemical analysis in a cohort of dogs with EHBO (n = 10).

Variable	Reference range	Median (range)	No. of dogs	
			Values above reference range	Values below reference range
Hct (%)	40.3–60.3	43.5 (29–47)	0	3
Neutrophil count ($\times 10^3/\mu\text{L}$)	3.1–14.4	14.6 (7.29–31.42)	6	0
Platelet count ($\times 10^3/\mu\text{L}$)	177–398	288 (107–468)	2	2
Total bilirubin (mg/dL)	0.1–0.5	8 (2.4–21)	10	0
Alkaline phosphatase (U/L)	20–155	2,837 (1,784–9,861)	10	0
Alanine aminotransferase (U/L)	16–91	1,212 (116–2,714)	9	0
γ -Glutamyl transferase (U/L)	7–24	55 (31–392)	9	0
Cholesterol (mg/dL)	128–317	420 (331–642)	9	0
Glucose (mg/dL)	65–112	95 (43–110)	0	2
BUN (mg/dL)	5–30	12 (7–19)	0	0
Albumin (g/dL)	2.5–3.7	2.9 (2.0–3.4)	0	2

Ten dogs with EHBO were evaluated; results for alanine aminotransferase and γ -glutamyl transferase activities and cholesterol, glucose, BUN, and albumin concentrations were available for 9 dogs.

Table 2—Coagulation test and thromboelastography results for 10 dogs with EHBO and 19 healthy control dogs.

Variable	Reference range	Control group	EHBO group
PT (s)	6.8–10.2	NA	8.35 (7.5–10.2)
APTT (s)	10.7–16.4	NA	13.7 (11.5–16.4)
Factor VII coagulant activity (%)	50–150	NA	64.5 (46–138)
Factor VIII coagulant activity (%)	50–200	NA	156 (115–500)
Factor XI coagulant activity (%)	60–150	NA	112 (71–270)
Protein C activity (%)	75–135	NA	89 (55–138)
D-dimer ($\mu\text{g/mL}$)	0.01–0.2	NA	0.78 (0.1–3.42)
Antithrombin activity (%)	65–145	71.6 \pm 14.2	74.9 \pm 9.2
Fibrinogen (mg/dL)	147–479	193.3 \pm 127.4	743.8 \pm 282.6*
R (min)	5–7.4	5.9 (2.3–13.6)	2.9 (1.7–5.6)*
K (min)	2.7–4.3	3.1 (1.5–7.9)	1.3 (0.8–2)*
α -Angle ($^\circ$)	44.1–55.8	48.7 \pm 11.4	71.5 \pm 6.2*
MA (mm)	53.7–61.3	56.8 \pm 7.7	70.6 \pm 9.3*
Coagulation index	1.3–2.4	1.7 \pm 1.2	5.3 \pm 1.4*

Data are reported as mean \pm SD or median (range).
 *Values differ significantly ($P < 0.001$) from those of control dogs.
 K = Clot formation time. NA = Not applicable. R = Reaction time.
 Because of a clerical error, factor VII coagulant activity was assayed in 5 dogs and factor VIII activity was assayed in the remaining 5 dogs of the EHBO group.

EHBO had a median K 1.7 minutes less (95% CI, 3.0 to 0.4) with outliers included and 1.4 minutes less (95% CI, 2.0 to 0.7; $P < 0.01$) with outliers excluded, mean α -angle 18.7° greater (95% CI, 9.6 to 27.8; $P < 0.001$), and mean MA 10.9 mm greater (95% CI, 3.3 to 18.6; $P = 0.007$), compared with values for control dogs.

There was no significant association between serum bilirubin concentration, plasma fibrinogen concentration, protein C activity, or Hct with coagulation index. The Hct was significantly ($P = 0.046$) associated with MA, whereas fibrinogen concentration was not. For every 5.0% increase in Hct, there was a 4.3% decrease in MA (95% CI, 8.5% to 0.1%).

Surgical findings and histopathologic results (EHBO group)—All dogs underwent a full exploratory celiotomy and were found to have either partial ($n = 2$) or complete (8) obstruction of the extrahepatic biliary tract. One dog with a gallbladder mucocele had a small tear in the cystic duct detected during flushing of the common bile duct. None of the other dogs had evidence of discontinuity of the extrahepatic biliary tract. In 2 dogs, an underlying neoplastic process was diagnosed, whereas in 8 dogs, no neoplastic processes were identified. One of the dogs with neoplasia had hepatic carcinoma, and the other had an anaplastic sarcoma in the region of the pancreas. Causes of EHBO in 8 dogs with nonneoplastic disease were gallbladder mucocele ($n = 2$), pancreatitis (1), fungal granuloma compressing the common bile duct (1), large cholelith in the common bile duct (1), and cholangiohepatitis (3, including the 2 dogs with partial obstruction). Seven of the 8 dogs with nonneoplastic disease had histologic examination of a liver sample, and abnormalities were detected in all of these: multiple small abscesses throughout the liver with fungal hyphae observed ($n = 1$), severe chronic active hepatitis (1), moderate chronic neutrophilic cholangitis (2), and various degrees of cholangiohepatitis (3). Further testing revealed that the dog with severe chronic hepatitis had a high hepatic copper concentration (2,180 $\mu\text{g/g}$; reference range, 105 to 400 $\mu\text{g/g}$). Seven of 10 dogs had samples collected at surgery for aerobic and anaerobic bacterial culture and susceptibility testing of the liver, bile, or gallbladder. In 6 of these, culture results were negative, and *Escherichia coli* and an *Enterococcus* sp were cultured from a liver sample from 1 dog. Results of all anaerobic cultures were negative.

Outcomes—Seven dogs were allowed to recover from surgery and were discharged from the hospital a median of 5 days (range, 2 to 13 days) after surgery. The dog with hepatic carcinoma was euthanized 2 days after surgery because of a deteriorating clinical condition. The owners of the dogs with anaplastic sarcoma and fungal granuloma elected euthanasia at the time of surgery because of poor prognosis.

Discussion

Canine patients with EHBO often require technically complex surgery to relieve obstruction. Among numerous physiologic derangements that must be considered, these patients are at risk for hemostatic imbalance. Although few studies reported in the veteri-

nary literature have evaluated hemostasis in dogs with EHBO, prolongation of the APTT was associated with a poor outcome in 1 retrospective case series.² An additional case report¹⁹ documented a vitamin K–dependent bleeding diathesis in a dog with complete EHBO secondary to stricture of the common bile duct.

Although considered a hypocoagulable state in dogs, EHBO typically manifests as a hypercoagulable state in humans.^{8–11} Thrombotic complications contribute to morbidity and death in humans with EHBO, and evidence of thrombosis has been noted at necropsy.²⁰ In cats and dogs, mortality rates associated with surgery of the extrahepatic biliary tract are very high.^{1–3} Although studies investigating thrombosis at necropsy in veterinary patients are lacking, it is possible that thrombosis plays a role in postoperative morbidity and death in these animals. Furthermore, the traditional preoperative coagulation screening tests, APTT and PT, are unsuited for detecting thrombosis or hypercoagulability. Recently, thromboelastography has been used as a more appropriate technique to investigate and characterize hypercoagulability in dogs with diseases such as immune-mediated hemolytic anemia,^{16,21} parvoviral enteritis,²² protein-losing nephropathy,¹⁵ and neoplasia.²³ To our knowledge, the present study is the first to evaluate dogs with EHBO by means of thromboelastography.

The most important finding of this study is that all 10 dogs with partial or complete EHBO were categorized as having hypercoagulability on the basis of thromboelastography, most notably high coagulation index values. Most dogs with EHBO also had abnormalities in R, K, α -angle, and MA consistent with hypercoagulability. In vivo, hypercoagulability implies hemostatic imbalance resulting from unopposed or systemic activation of coagulation factors and platelets, loss or downregulation of physiologic anticoagulants, or reduced fibrinolysis.²⁴ A number of mechanisms have been proposed to account for hypercoagulability in human patients with EHBO. These include biliary tract infection and sepsis or endotoxemia initiating DIC,²⁰ procoagulant effects of endotoxemia,²⁵ and cholestasis-induced platelet hyperreactivity.^{10,11}

Although our study provided evidence of hypercoagulability in dogs with EHBO, similar to the human disease counterpart, the underlying mechanism cannot be established from these data. Disseminated intravascular coagulation was considered unlikely because few dogs met the clinical or laboratory criteria of this syndrome. Specifically, PT and APTT were within respective reference ranges in all affected dogs; none had a low plasma fibrinogen concentration, only 1 had low antithrombin activity, and only 2 had mild thrombocytopenia. Bacteremia or endotoxemia cannot be definitively ruled out as contributory factors; however, only 1 of the 7 dogs tested in this study had positive results of bacterial culture. Specific tests of platelet activation status or platelet reactivity were not performed; therefore, the potential role of platelets in mediating hypercoagulability could not be evaluated.

Hyperfibrinogenemia and high D-dimer concentration were the most consistent coagulation assay abnormalities (detected in 7 and 8 dogs, respectively) in the present study. Fibrinogen is an acute-phase protein

synthesized by the liver; its concentration is typically increased in dogs with sustained or systemic inflammation. The high proportion of dogs with EHBO that had hyperfibrinogenemia is an indicator of the inflammatory nature of this disorder. Considering that MA and coagulation index are dependent on plasma fibrinogen content,¹² the moderate to marked increase in plasma fibrinogen concentration in 7 of 10 dogs with EHBO may have contributed to the hypercoagulability as assessed by thromboelastography, although there was not a significant association between fibrinogen concentration and MA or coagulation index in our study. Results of numerous epidemiological studies^{26,27} in humans have indicated positive associations between plasma fibrinogen concentration and arterial and venous thrombosis. Recently, a causal role has been demonstrated for hyperfibrinogenemia in promoting rapid, fibrin-dense, and fibrinolysis-resistant thrombus formation in mice used to study vascular injury.²⁸ Hyperfibrinogenemia in dogs with EHBO may ultimately represent a risk factor for thrombosis and not just a biomarker of an ongoing inflammatory process.

High plasma D-dimer concentration is a marker of intravascular fibrin formation and secondary fibrinolysis but is not specific for thrombosis or thromboembolic disease. High D-dimer concentrations have been detected in dogs undergoing various surgical procedures or having internal hemorrhage, liver disease, and neoplasia.^{29,30} In particular, D-dimer concentration may have limited utility as a diagnostic indicator of thrombosis in patients with liver disease. These patients may have high D-dimer concentration due to enhanced fibrinolysis (caused by impaired hepatic synthesis of inhibitors of fibrinolysis and delayed hepatic clearance of tissue plasminogen activator) and impaired hepatic clearance of D-dimer,³¹ rather than secondary to increased fibrin formation. Nevertheless, in 1 study,²⁹ markedly high plasma D-dimer concentrations (to > 1 µg/mL) were found to have a 94% specificity for thromboembolic disease in dogs. In that study,²⁹ 8 of 10 dogs with EHBO had D-dimer concentrations above the reference range, with 5 of the 8 having markedly high concentrations (ie, > 1 µg/mL) suggestive of a thrombotic process. Similarly, we found a high proportion of EHBO-affected dogs with high plasma D-dimer concentration (8/10) in our study. The concomitant presence of liver disease and cancer in our patient population complicated the interpretation of these results. However, a markedly high D-dimer concentration may be indicative of subclinical or incipient thrombosis.

Deficiencies of antithrombin and protein C activities were uncommon in the EHBO-affected dogs of the present study, ruling out anticoagulant deficiency as the cause of hypercoagulability determined via thromboelastography in this group. These plasma anticoagulants play a critical role in regulating thrombin formation. Both are synthesized in the liver, and deficiencies of these proteins have been described in dogs with various forms of liver disease.³² Dogs with EHBO were considered at additional risk for a functional protein C deficiency due to inadequate intestinal vitamin K absorption to support γ -carboxylation and subsequent activation of the protein. However, none of the dogs with EHBO had

prolonged PT or APTT, indicating sufficient vitamin K absorption to support posttranslational processing and function of vitamin K–dependent coagulation.

Liver function and underlying disease processes were further characterized by results of blood and serum biochemical analyses, coagulation factor assays, and histologic examination of biopsy samples. Mild reduction in protein C activity was found in 3 of 10 dogs, providing evidence of some hepatic compromise; however, the circulating BUN concentrations were within reference ranges in all 9 dogs tested, and albumin and glucose concentrations were decreased in only 2 of 9 dogs. Although low albumin concentration may result from failure of hepatic synthesis, concomitant hyperfibrinogenemia in dogs with EHBO suggested that albumin synthesis may have been specifically downregulated in an acute-phase response. With the exception of 1 dog with moderately low factor VII coagulant activity, dogs with EHBO had factor activities within or above the reference range. This finding also indicated adequate hepatic synthetic capacity. Factor VIII is an acute-phase protein, and activation of the contact pathway and factor XI secondary to endotoxemia has been described in humans.^{33,34} Histologic evaluation of liver samples provided direct evidence of neoplastic, infectious, or sterile inflammatory processes with various degrees of severity in all dogs with EHBO that were examined. In light of these findings, it is unlikely that the dogs with EHBO in this study had clinically relevant hepatic synthetic failure.

There were several limitations to the study reported here. The number of dogs included in the EHBO group was small, primarily because of the infrequent nature of this condition in the canine population. Despite a thorough search of the medical records database, no homogeneous control population consisting of dogs with noncholestatic liver disease that had undergone thromboelastographic evaluation was available. Such a population may have allowed further evaluation of the relationship between cholestatic and noncholestatic disease states with regard to coagulation.

Clearly, some of the dogs with EHBO had some degree of liver dysfunction, which influenced results of some tests. The control population used in this study was significantly younger and consisted of a greater number of mixed-breed dogs, compared with the EHBO population, which may have also influenced some of the results of coagulation tests.

The population of dogs with EHBO in our study was not homogeneous with respect to underlying cause, and it was not possible to determine the duration of EHBO prior to evaluation. In addition, because of the life-threatening nature of EHBO and requirement for emergency surgery, dogs were not comprehensively evaluated for concurrent disorders (eg, hyperadrenocorticism or protein-losing nephropathy) that may have contributed to the hypercoagulability. The thromboelastography assay was performed with nonactivated (as opposed to kaolin- or tissue factor–activated) blood samples; the lack of trigger reagents may increase variability in K. However, all control and EHBO samples were handled and assayed in the same manner to minimize preanalytic variability.

A correlation between Hct and MA (but not coagulation index) was found in this study. Recently, an effect of anemia on thromboelastography variables has been described by other investigators,^{j,k} and this effect may have influenced results of the present study. Three of 10 dogs with EHBO in our study were anemic, potentially yielding more hypercoagulable thromboelastography results than would have been detected in blood samples with normal Hct.^{j,k}

Because the liver has a central role in synthesizing hemostatic and fibrinolytic pathway proteins, patients with hepatic disease often have complex coagulopathies. Although systemic inflammation represents a prothrombotic stimulus,^{35,36} further studies will be required to clarify the role of inflammation in the pathophysiology of hypercoagulability in dogs with EHBO. Platelet function testing would help determine whether enhanced platelet activation contributes to hypercoagulability in these patients. Evaluating thromboelastography and other coagulation variables after surgery would demonstrate the effect of reestablishment of bile flow on these variables in dogs treated for EHBO. This would be particularly interesting because results in human patients with EHBO have yielded conflicting evidence regarding postoperative resolution of hypercoagulability.^{11,20}

The clinical importance of this study lies in the fact that hypercoagulability as assessed by thromboelastography was frequently detected among dogs with EHBO. The traditional view of EHBO as a disease that causes hypocoagulability should be reconsidered in light of these findings.

- a. Cell-Dyn 3700, Abbott Diagnostics, Abbott Park, Ill.
- b. Vitros 350 Chemistry System, Ortho-Clinical Diagnostics, Rochester, NY.
- c. STA Compact, Diagnostica Stago, Parsippany, NJ.
- d. Accuclot D-dimer, Sigma Diagnostics, St Louis, Mo.
- e. TEG 5000 Analyzer, Haemoscope Corp, Niles, Ill.
- f. Stachrom ATIII, Diagnostica Stago, Parsippany, NJ.
- g. Stachrom Protein C, Diagnostica Stago, Parsippany, NJ.
- h. StaCompact, Diagnostica Stago, Parsippany, NJ.
- i. Stata II, Statocorp LP, College Station, Tex.
- j. Jaquith SD, Brown AJ, Scott MA. Effects of decreased hematocrit on canine thromboelastography (abstr). *J Vet Emerg Crit Care* 2009;19(suppl 1):A4.
- k. Smith SA, McMichael M, Galligan A, et al. Whole blood thromboelastometry (TEM) is related to cell counts and plasma coagulation tests in healthy dogs (abstr). *J Vet Intern Med* 2009;23:692.

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From this month's AJVR

Comparison of first-intention healing of carbon dioxide laser, 4.0-MHz radiosurgery, and scalpel incisions in ball pythons (*Python regius*)

Rebecca T. Hodshon et al

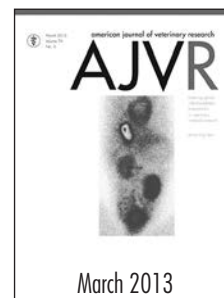
Objective—To evaluate first-intention healing of CO₂ laser, 4.0-MHz radiowave radiosurgery (RWRS), and scalpel incisions in ball pythons (*Python regius*).

Animals—6 healthy adult ball pythons.

Procedures—A skin biopsy sample was collected, and 2-cm skin incisions (4/modality) were made in each snake under anesthesia and closed with surgical staples on day 0. Incision sites were grossly evaluated and scored daily. One skin biopsy sample per incision type per snake was obtained on days 2, 7, 14, and 30. Necrotic and fibroplastic tissue was measured in histologic sections; samples were assessed and scored for total inflammation, histologic response (based on the measurement of necrotic and fibroplastic tissues and total inflammation score), and other variables. Frequency distributions of gross and histologic variables associated with wound healing were calculated.

Results—Gross wound scores were significantly greater (indicating greater separation of wound edges) for laser incisions than for RWRS and scalpel incisions at all evaluated time points. Necrosis was significantly greater in laser and RWRS incisions than in scalpel incision sites on days 2 and 14 and days 2 and 7, respectively; fibroplasia was significantly greater in laser than in scalpel incision sites on day 30. Histologic response scores were significantly lower for scalpel than for other incision modalities on days 2, 14, and 30.

Conclusions and Clinical Relevance—In snakes, skin incisions made via a scalpel generally had less necrotic tissue than did CO₂ laser and RWRS incisions. Comparison of the 3 modalities on the basis of histologic response scores indicated that use of a scalpel was preferable, followed by RWRS and then laser. (*Am J Vet Res* 2013;74:499–508)



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