Evidence of hypercoagulability in dogs with parvoviral enteritis

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Objective—To determine whether dogs with naturally occurring canine parvoviral (CPV) enteritis have laboratory evidence of hypercoagulability.

Design—Case-control study.

Animals—9 dogs with naturally occurring CPV enteritis and 9 age-matched control dogs.

Procedure—Blood was collected from all dogs within 24 hours of admission for thromboelastography (TEG) and determination of activated partial thromboplastin time (aPTT), prothrombin time (PT), antithrombin III (AT) activity, and fibrinogen concentration. Fibrin-fibrinogen degradation product (FDP) concentration, D-dimer concentration, and platelet count were obtained in dogs with CPV enteritis only. Records were reviewed for evidence of thrombosis or phlebitis.

Results—All 9 dogs with CPV enteritis had evidence of hypercoagulability, determined on the basis of significantly increased TEG maximum amplitude and decreased AT activity. Fibrinogen concentration was significantly higher in dogs with CPV enteritis than in control dogs. The aPTT was moderately prolonged in dogs with CPV enteritis, and FDP concentration was < 5 mg/ml in 7 of 9 dogs. No dogs had a measurable D-dimer concentration. Platelet counts were within reference range. Four of 9 dogs had clinical evidence of venous thrombosis or phlebitis associated with catheters. One dog had multifocal splenic thrombosis identified at necropsy.

Conclusions and Clinical Relevance—Dogs with CPV enteritis have a high prevalence of clinical thrombosis or phlebitis and laboratory evidence of hypercoagulability without disseminated intravascular coagulopathy. Thromboelastography may help identify hypercoagulable states in dogs. (J Am Vet Med Assoc 2000;217:1500–1504)

Canine parvoviral (CPV) enteritis is a common disease that affects young dogs. Clinical signs of vomiting and diarrhea are associated with viral destruction of rapidly dividing cells of the intestine and bone marrow. Mortality associated with this disease, however, appears to result from bacteremia and endotoxemia. In one study, 82% of dogs with CPV enteritis had measurable endotoxin in circulation. Endotoxin is a potent stimulus for the inflammatory response through activation of cytokine-mediated (eg, tumor necrosis factor) procoagulant effects on endothelial cells. The initial response to endotoxin is activation of coagulation. This response, however, is not well characterized by assays that measure in vitro clotting time, because these tests are primarily sensitive to coagulation factor deficiencies or factor inhibition. As the inflammatory response progresses, systemic hypercoagulability may progress to hypocoagulability, with fulminant signs of hemorrhage recognized as disseminated intravascular coagulation (DIC).

A predisposition for formation of thrombi in response to stimuli or injury that would not normally result in thrombosis is referred to as a hypercoagulable state. Virchow originally proposed that thrombus formation resulted from a combination of abnormal coagulation resulting in inappropriate or excessive platelet or fibrin deposition (hypercoagulability), vascular injury, and blood stasis. Hypercoagulability may result from increased activation of coagulation factors and platelets, decreased anticoagulants, or decreased fibrinolysis. Although there is no gold standard for the diagnosis of hypercoagulability, there are reports of different methods that attempts to identify hypercoagulability. One of the most promising methods is thromboelastography (TEG), a technique that characterizes coagulation function as an integrated unit.

Originally developed by Hart et al in 1948, TEG creates a tracing that represents the formation and breakdown of a blood clot. Different thromboelastographic patterns have been associated with a variety of hemostatic disorders, including clotting factor deficiency, thrombocytopenia, fibrinolysis, and hypercoagulability. Thromboelastography has been used to identify the hypercoagulable state that precedes the development of clinically recognizable DIC in human patients with sepsis. In veterinary medicine, TEG has been evaluated in dogs and cats. On the basis of the development of catheter-related thrombosis or phlebitis in 8 of 9 dogs with CPV enteritis prior to the initiation of the study reported here, we hypothesized that dogs with clinical CPV enteritis had activation of coagulation leading to hypercoagulability early in the course of their disease. The purpose of the study reported here was to determine whether dogs with naturally occurring CPV enteritis have laboratory evidence of hypercoagulability.
Materials and Methods

Dogs—Nine client-owned dogs that were 9 weeks to 8 months old were enrolled in this study. Enrollment criteria included positive results for a fecal CPV antigen test—a clinical signs of vomiting, diarrhea, or both for 1 to 4 days prior to admission, signed client consent, admission to the Veterinary Hospital at the University of Pennsylvania as part of a clinical trial, and no evidence of DIC. All dogs had a complete physical examination and received supportive treatment (IV administration of fluids, antimicrobials) for the treatment of CPV enteritis. The control group consisted of 9 dogs that were age-matched to the 9 dogs with CPV enteritis. Control dogs were selected from a closed research colony that was free of parvovirus infection and maintained according to National Institutes of Health (NIH) guidelines. The dogs were determined to be healthy on the basis of results of physical examination. The Animal Use Committee of the University of Pennsylvania approved the sample collection protocol.

Sample collection—Two tubes of citrated blood (2 ml/tube; 3.8% citrate in 9:1 blood-to-citrate ratio) were obtained from all dogs either from a clean venipuncture by use of an evacuated-tube collection system in the control dogs or from a catheter that was placed in the jugular vein by use of a 2-syringe collection method in dogs with CPV enteritis. In 2 dogs with CPV enteritis, catheter and venipuncture samples obtained simultaneously were evaluated; TEG tracings were not affected by route of collection, so subsequent samples were all drawn from catheters. An initial aliquot of blood (approx 2 ml) was discarded from each dog, then the first sample was drawn, placed on ice, and centrifuged; plasma was removed and frozen at -70 C within 10 minutes for batch coagulation testing within 1 month. The sample was overlaid with oil, and the computerized tracing initiated. Measurements obtained from each sample (Fig 1) included R value (time from start of the assay to an amplitude of 2 mm [this amplitude indicates initial fibrin formation]a), K (time from the endpoint of the R time to a fixed point of clot firmness when the amplitude of the tracing was 20 mm wide [this amplitude represents platelets and fibrinogen]), maximum amplitude (MA; the greatest amplitude of the TEG tracing, which reflects the absolute strength of the clot), and alpha angle (Ang; the slope of the TEG tracing, which indicates rate of clot formation). The aPTT reagent was used with an activation time of 180 seconds. The PT was performed by use of a rabbit brain phospholipid reagent." Fibrinogen assay was performed via the Clauss method by use of a human thrombin reagent (100 NIH U/ml) and a standard curve derived from dilutions of pooled normal canine plasma (386 mg of fibrinogen/dl). The fibrinogen concentration of the canine plasma standard was determined by use of a quantitative multispecies fibrinogen ELISA. Plasma AT activity was measured by use of a synthetic chromogenic substrate kit according to the manufacturer's recommendations for assay method and instrumentation. A standard curve was derived from dilutions of pooled normal canine plasma that had an assigned value of 100% AT activity. Antithrombin of test plasma samples was reported as a percentage of the activity of the pooled normal canine plasma standard. Plasma concentrations of FDP and D-dimer were measured by use of commercial latex agglutination kits according to manufacturer's instructions.

![Figure 1](https://example.com/figure1.png)

Figure 1—Thromboelastographic (TEG) tracing obtained from analysis of a blood sample from a clinically normal 4-month-old puppy. Standard chart speed (2 mm/min) was used. R = Reaction time, measured from the start of the tracing to the point where the divergent arms of the TEG tracing reach an amplitude of 1 mm. K = Coagulation time, measured from the point where the amplitude of the TEG is 1 mm to the point where the amplitude of the divergent arms of the tracing equals 20 mm. Ang = Angle (degrees [deg]) measured between the midline of the tracing and a line drawn from the 1-mm point tangential to the curve, indicating the slope of the tracing. MA = Maximum amplitude, the width of the curve at its widest point.
Platelet counts—Platelet counts from dogs with CPV enteritis were obtained from blood samples collected into tubes containing EDTA on the same day as the TEG samples. Platelet counts were measured by use of an automated counter.

Medical record review—Medical records were reviewed in a retrospective manner to identify clinical or histologic evidence of thrombosis or phlebitis. Plasma total solids concentration and serum albumin concentration, when available, were recorded.

Statistical analyses—The TEG values, aPTT, PT, AT activity, and fibrinogen concentration from dogs with CPV enteritis were compared with those of age-matched control dogs by use of a paired t-test. Significance was set at \( P < 0.05 \). The effect of platelet count and fibrinogen concentration on MA was evaluated by use of linear regression analysis. Results were expressed as mean ± SD unless indicated otherwise.

Results

Nine dogs were enrolled in the study. Blood samples obtained from the 9 dogs were judged to be hypercoagulable on the basis of increased MA (78.3 ± 4.6 mm vs 62.0 ± 8.0 mm in control dogs; \( P < 0.001 \); Fig 2) and decreased AT activity (72.2 ± 8.7% vs 84.1 ± 9.0% in control dogs; \( P = 0.003 \)). Fibrinogen concentration was significantly (\( P < 0.001 \)) higher in dogs with CPV enteritis (1,119 ± 325 mg/dl) than in age-matched control dogs (437 ± 96 mg/dl). The aPTT was moderately prolonged in dogs with CPV enteritis (22.8 ± 5.7 seconds vs 16.3 ± 0.8 seconds in control dogs; \( P = 0.013 \)). The FDP concentration was < 5 mg/ml in 7 of 9 dogs with CPV enteritis, and none of these dogs had measurable D-dimer concentrations. Platelet counts of dogs with CPV enteritis ranged from 186,000 to 473,000/m\(^3\) (mean, 334,000 ± 98,000 platelets/m\(^3\)), and all counts were within or above the laboratory reference range (175,000 to 400,000 platelets/m\(^3\)). Samples from dogs with CPV enteritis had slightly reduced R value, compared with control dogs (10.2 ± 4.7 vs 15.1 ± 8.6), but the difference between groups was not significant (\( P = 0.17 \)). Significant difference between groups was not detected for the K or A values, but variation in the values was high. The PT values were not significantly different between groups. Significant associations were not detected between platelet count and MA or between fibrinogen concentration and MA in dogs with CPV enteritis.

Four of 9 dogs developed clinical evidence of venous thrombosis or phlebitis associated with catheters. One dog had multifocal splenic thrombosis identified at necropsy.

Mean plasma total solids concentration in dogs with CPV enteritis was 5.2 ± 0.6 mg/dl after approximately 24 hours of supportive care (day 1). The mean day-1 serum albumin concentration, in the 4 dogs in which it was measured, was 1.8 ± 0.2 g/dl and accounted for only 25% of the concentration of total solids.

Discussion

We observed catheter-related thrombosis or phlebitis in 8 of 9 dogs with CPV enteritis that were treated prior to the initiation of the study reported here. Thus, on the basis of reports of hypercoagulability associated with sepsis' and the known association of endotoxemia and activation of the cytokine cascade with CPV infection, we evaluated dogs with CPV enteritis for evidence of hypercoagulability. Because routine coagulation screening tests are not reliable indicators of hypercoagulability, we performed additional tests to detect systemic activation of coagulation. Thromboelastography is a tool that provides an integrated evaluation of clotting function. The tracing depends on the interactions of coagulation factors, platelets, fibrin, fibrinolysis, and time. All 4 of the variables measured by use of TEG in our study may be altered by hypercoagulability. Hypercoagulability may cause reduced R and K values, increased MA, and increased Ang. Coagulation is a complex process with a vast number of interdependent reactions.

![Figure 2](image-url) —Thromboelastographic tracing obtained from analysis of a blood sample from a 4-month-old puppy with canine parvoviral enteritis. See Figure 1 for key.
between platelets, WBC, endothelial cells, and fluid-phase hemostatic proteins. Because of this complexity, in vitro coagulation screening tests designed to detect factor deficiencies are not good predictors of an in vivo hypercoagulable state. Thromboelastography provides a more physiologic and global test of hemostasis. The TEG tracing portrays not only clot formation but also the structural characteristics of the formed clot and its stability.

Results of the study reported here indicate that puppies with CPV enteritis have increased MA, compared with that of age-matched control dogs. In humans, tracings with MA values > 70 mm are considered indicative of a hypercoagulable state. Eight of 9 dogs with CPV enteritis in our study had MA values > 70 mm, whereas all control dogs had MA values ≤ 70 mm. Lack of significant differences between the 2 groups with regard to other variables was likely a result of the large variation among values and the small sample numbers. High fibrinogen concentrations and high platelet counts may contribute to a widening of the MA. We did not detect a direct relationship between the MA and the fibrinogen concentration or the MA and the platelet count in dogs with CPV enteritis. A more complex relationship among these factors may exist.

Reductions in plasma AT activity to 50 to 75% of values measured in clinically normal dogs are associated with a moderate predisposition to thrombosis. In our study, AT activity was significantly lower in dogs with CPV enteritis, and 6 of 9 dogs had AT activity < 75% of the activity of the pooled standard. There are 3 potential explanations for low AT activity: increased AT loss, dilution of AT, or AT consumption. It has been suggested that albumin concentrations may mirror AT protein concentrations in conditions resulting in protein loss (eg, protein-losing nephropathy). Albumin measurements were only available in 4 dogs with CPV enteritis; in those dogs, albumin only accounted for 25% of total solids rather than the expected proportion of 35 to 50%. This disproportion may be explained by the high fibrinogen concentrations in dogs with CPV enteritis. The low total solids and associated low albumin in dogs with CPV enteritis suggest that gastrointestinal protein loss or protein dilution related to fluid therapy may contribute to the low AT activity. A third explanation for low AT activity is consumption as the result of endotoxin-mediated activation of coagulation. The prolonged aPTT could have resulted from endotoxin-mediated activation of coagulation factors or dilution of coagulation factors. The aPTT is more sensitive to subtle changes in coagulation factor activity than PT, and consumption of prekallikrein by cytokine-initiated conversion to kallikrein may have contributed to the findings we detected in dogs with CPV enteritis. Coagulopathy or low AT activity caused by dilution, however, would be expected to be accompanied by TEG tracings that reveal a decrease in MA. The dogs with CPV enteritis in our study had high MA (Fig 2).

Dogs with CPV enteritis had significant increases in fibrinogen concentration. The inflammatory response initiated by the viral disease and associated endotoxemia were likely causes of this increase. Increases in fibrinogen concentration have been reported in dogs with CPV infection. Although not predictive of thrombosis, increased fibrinogen concentration in association with vascular stasis, activation of coagulation, and vascular injury (Virchow's triad) may be a risk factor for thrombosis and contribute to the hypercoagulable state in these dogs.

Measurement of D-dimer concentration (a degradation product of cross-linked fibrin) has been advocated as a diagnostic tool for thrombotic states. It may also be valuable as a marker of hypercoagulability. Although diagnostic sensitivity of this test for the diagnosis of thrombosis in humans (eg, deep vein thrombosis, pulmonary thromboembolism) is high, the sensitivity for identification of dogs at risk for thrombosis is unknown. Clearly, in dogs with CPV enteritis in our study, which did not have clinically evident thrombosis at the time of testing, the D-dimer test did not identify a hypercoagulable state. The finding of high plasma FDP concentration, in the absence of high D-dimer concentration, may be caused by fibrinogenolysis attributable to unopposed or unregulated plasmin activity (without a comitant increase in thrombin activity) or fibrinogenolysis mediated by nonplasmin proteinases (eg, polymorphonuclear elastase).

Discordant FDP and D-dimer results may also result from differential test sensitivities; according to the manufacturer, the D-dimer assay is less sensitive than the FDP assay.

The small number of dogs was a limiting factor in the study reported here, but the findings were important and in accordance with clinical catheter-related thrombosis in dogs with CPV enteritis. Although we cannot define the primary mechanism, results of our study suggest that reduction in AT activity and increase in fibrinogen concentration are likely to contribute to early development of hypercoagulability in dogs with CPV enteritis. Supportive treatment by administration of fresh-frozen plasma may provide AT as well as immunoglobulins and albumin. It is likely, however, that hypercoagulability is the result of several factors, including high fibrinogen concentration, and transfusion could exacerbate signs through further increases in plasma fibrinogen concentration.

References


