Coagulation status, fibrinolysis, and platelet dynamics in dogs with chronic inflammatory enteropathy

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Abstract

Background: Coagulation status is poorly understood in dogs with chronic inflammatory enteropathy (CIE). Fibrinolytic activity and platelet dynamics have not been evaluated in CIE dogs.

Objectives: To assess coagulation status and fibrinolysis in normoalbuminemic CIE dogs (CIE-N) and CIE dogs with protein-losing enteropathy (CIE-PLE) compared to healthy controls (HC). To evaluate thromboelastography (TEG) variable differences between groups and for correlations with clinicopathologic data. To report platelet dynamics in CIE dogs.

Animals: Twenty-five client-owned dogs with CIE (n = 16 CIE-N; n = 9 CIE-PLE); 14 HC beagle dogs.

Methods: All dogs had tissue factor + tissue plasminogen activator TEG. Nine of 25 CIE dogs had whole blood impedance platelet aggregometry. The TEG variables and coagulation data were compared between all CIE vs HC dogs, CIE-N dogs vs HC, and CIE-PLE dogs vs HC. Clinicopathologic and coagulation data were available for CIE dogs and assessed for correlation to TEG variables.

Results: Dogs with CIE had higher maximum amplitude (MA; P < .001), longer clot lysis times (CLTs; P < .001), lower % lysis after 30 minutes (LY30; P < .001), and % lysis after 60 minutes (LY60; P < .001) compared to HC, suggesting hypercoagulability and hypofibrinolysis. When separated out, both CIE-N and CIE-PLE dogs had higher MA, longer CLT, and lower LY30 and LY60 compared to HC. Serum albumin and 25-hydroxyvitamin D (25[OH]D) concentrations, and plasma antithrombin and fibrinogen concentrations moderately correlated with MA.

Conclusions and Clinical Importance: Normoalbuminemic and hypoalbuminemic CIE dogs were considered hypercoagulable based on TEG compared to HC. Some CIE dogs displayed hypofibrinolytic phenotypes on TEG.

Abbreviations: 25[OH]D, serum 25-hydroxyvitamin D; AA, arachidonic acid; ADP, adenosine diphosphate; aPTT, activated partial thromboplastin time; AT, antithrombin; AUC, area under the curve; CCECAI, canine chronic enteropathy clinical activity index; CIE, chronic inflammatory enteropathy; CIE-N, normoalbuminemic CIE dogs; CIE-PLE, CIE dogs with protein-losing enteropathy; CLT, clot lysis time; HC, healthy controls; IBD, inflammatory bowel disease; IFA, immunofluorescent antibody testing; LY30%, lysis after 30 minutes; LY60%, lysis after 60 minutes; MA, maximum amplitude; PLE, protein-losing enteropathy; RI, reference interval; TAFI, thrombin-activatable fibrinolysis inhibitor; TE, thromboembolism; TEG, thromboelastography; TF, tissue factor; tPA, tissue plasminogen activator.
1 | INTRODUCTION

Dogs with protein-losing enteropathies (PLEs) secondary to chronic small intestinal disease are at risk of developing life-threatening thromboembolism (TE) and display a hypercoagulable phenotype on thromboelastography (TEG). Similarly, TE is a well-recognized complication of inflammatory bowel disease (IBD) in humans, with IBD patients having a reported risk of TE 3 times higher than that of the general population. Although both normoalbuminemic and hypoalbuminemic humans with IBD are at risk for TE, it is unclear whether dogs with chronic inflammatory enteropathy (CIE) without secondary PLE display a hypercoagulable phenotype on TEG.

Although it has long been recognized that inflammation promotes a prothrombotic state, the exact pathogenesis of hypercoagulability in humans with IBD is not well understood. Acquired risk factors include immobilization, corticosteroid treatment, active disease, cobalamin and folate deficiencies, and hyperhomocysteinemia. Importantly, cobalamin and folic acid deficiencies are well described in dogs with CIE and hyperhomocysteinemia has been identified in cobalamin-deficient dogs. Therefore, these factors may play a role in the pathogenesis of hypercoagulability in dogs with chronic small intestinal disease, analogous to what is described in humans. Hypoalbuminemia also has been identified as a risk factor for thromboembolic events in hospitalized human patients with IBD. Similarly, dogs with hypoalbuminemia secondary to chronic small intestinal disease are reported to develop TE. Recently, a role for vitamin D deficiency in the development of TE has been postulated, which is noteworthy because vitamin D deficiency is common in humans with IBD. Decreased serum 25-hydroxyvitamin D (25(OH)D) concentrations have been reported in dogs with chronic gastrointestinal disease, especially dogs with PLE.

Alterations of the coagulation system identified in humans with IBD include decreases in anticoagulant factors, increases coagulation factors, increased platelet aggregation, hypofibrinolysis, and alterations in TEG variables. Platelet aggregation and fibrinolysis have not been evaluated in dogs with CIE with and without PLE. Although TEG has been used to document hypercoagulability in dogs with PLE, the mechanisms underlying the hypercoagulability remain unknown. Decreased antithrombin (AT) and increased fibrinogen concentrations have been reported in hypercoagulable dogs with PLE, but not specifically assessed in dogs with CIE without secondary PLE, or in correlation with TEG variables. Because TEG is not widely available to veterinarians, identifying potential associations between TEG parameters and other clinical indicators at the veterinarian’s disposal (eg, AT, fibrinogen, cobalamin, 25(OH)D) would be useful.

Thus, our objectives were to (a) utilize TEG to assess coagulation status and fibrinolysis in normoalbuminemic and hypoalbuminemic dogs with CIE compared to healthy controls (HC), (b) to assess for correlations between TEG data and a variety of clinicopathologic and hemostatic data to evaluate for possible markers of hypercoagulability, and (c) report platelet aggregometry findings in dogs with CIE.

2 | MATERIALS AND METHODS

2.1 | Study population—CIE dogs

Dogs presented to Colorado State University Veterinary Teaching Hospital for evaluation of chronic gastrointestinal signs (eg, decreased appetite, vomiting, diarrhea, weight loss) of at least 3 weeks’ duration were screened for inclusion in the study. Dogs were eligible for inclusion if they underwent a comprehensive diagnostic evaluation to exclude nongastrointestinal and other relevant gastrointestinal illness and culminating with a histologic diagnosis of small intestinal disease characterized by inflammatory infiltrates and morphologic changes. Dogs with evidence of intestinal neoplasia or infectious enteropathies (eg, intestinal histoplasmosis) were not eligible for inclusion. All small intestinal samples for histology were obtained endoscopically, and 22/25 dogs had both duodenum and ileum available for histologic evaluation. The remaining 3 dogs had only duodenal samples available for histologic evaluation. As part of their comprehensive evaluation, all dogs had hematology and serum biochemistry profiles performed. All dogs had serum albumin, cobalamin, folate, and 25(OH)D (DACPAH; MSU Diagnostic Center for Population and Animal Health, Meridian Charter Township, Michigan) concentrations determined. Routine abdominal ultrasonography was performed in all dogs by or under the supervision of a board-certified veterinary radiologist to evaluate for extra-intestinal disease or extra-luminal intestinal masses before endoscopic examination. Furthermore, all dogs had exocrine pancreatic insufficiency excluded by measurement of fasted serum trypsin-like immunoreactivity >5.0 mg/mL and hypoadrenocorticism excluded by basal serum cortisol concentrations >2 µg/mL or normal response to ACTH stimulation. The feces of all dogs were screened for helminths (fecal floatation), Giardia (immunofluorescent antibody testing [IFA]), and Cryptosporidium (IFA), with no parasites detected in any case. Finally, at the time of enrollment, a canine chronic enteropathy clinical activity index (CCECAI) score was calculated for each dog using serum albumin concentration, presence or absence of peritoneal effusion on ultrasound examination and the owner’s scores on appetite, activity level, vomiting, fecal consistency, fecal frequency, weight loss, and pruritus.

All tests were performed within 1 week of hemostatic testing and endoscopy. Hemostatic testing was performed on the day before or morning of the endoscopic procedure, before anesthesia. However, inclusion in the study ultimately required histologic evidence of CIE as described above.
Dogs were placed in the CIE dogs with PLE (CIE-PLE) group if serum albumin concentration was <2.5 g/dL. Urinalysis with or without urine protein: creatinine ratio and fasting and postprandial bile acid concentrations were performed to exclude other causes of hypoalbuminemia in dogs with serum albumin concentration <2.5 g/dL. Hypoalbuminemic dogs were required to have no clinically relevant proteinuria (negative urine dipstick test result or urine protein: creatinine ratio < 0.5) and no evidence of clinically relevant hepatic disease based on normal fasted with or without postprandial bile acid concentrations. Dogs being treated with medications known to affect coagulation (e.g., nonsteroidal anti-inflammatory drugs, thomboprophylactic drugs, corticosteroids, vitamin K) or with concurrent diseases known to be associated with coagulation derangements (e.g., hyperadrenocorticism, immune-mediated hemolytic anemia, extra-hepatic neoplasia) were excluded. All owners whose dogs were enrolled in the study gave informed consent. The study was approved by the Clinical Review Board at Colorado State University.

2.2 | Hemostatic analysis

All blood samples were collected by licensed veterinary technicians or study investigators from the jugular vein using a syringe with attached 20-gauge needle. Blood was collected for whole blood impedance platelet aggregometry (9/25 dogs; Multiplate 5.0 Analyzer, Diapharma Group, Inc, West Chester, Ohio), tissue factor (TF)-activated TEG (TEG 5000 Thrombelastograph Hemostasis Analyzer, Haemoscope Corporation, Braintree, Massachusetts), 1-stage prothrombin time, activated partial thromboplastin time (aPTT), quantitative fibrinogen (Clauss method), AT activity, and D-dimers. One-stage prothrombin time, aPTT, quantitative fibrinogen, and D-dimer assays were performed using the AMAX Destiny Plus (TCoaG, Stago Group, Asnieres-sur-Seine, France). Antithrombin activity was measured using a factor II-dependent assay on the AMAX Destiny using manufacturer recommended protocols. Briefly, residual thrombin activity after the addition of a thrombin/heparin reagent (TriniCHROM Antithrombin Ila, TCoaG, Stago Group, Asnieres-sur-Seine, France) and a thrombin substrate was measured chromogenically. The distribution of data for statistical analysis was assessed using the Shapiro-Wilk test. Normally distributed (parametric) variables were compared before each individual TEG analysis.

2.3 | Healthy control population

Hemostatic data including the results of TF-tPA TEG as described above were available from 14 healthy beagle dogs. Beagle dogs were historical controls and were not specifically age- or sex-matched for our study. Before data collection, all dogs had a physical examination, CBC, serum biochemistry profile, and urinalysis performed to establish their suitability to serve as healthy controls.

2.4 | Statistical analysis

Comparisons were performed among TEG variables for CIE vs HC dogs, CIE-N vs HC dogs, and CIE-PLE vs HC dogs. The CIE dogs were defined as CIE-PLE if their serum albumin concentration was <2.5 g/dL and CIE-N if their serum albumin concentration was ≥2.5 g/dL. The distribution of data for statistical analysis was assessed using the Shapiro-Wilk test. Normally distributed (parametric) variables were
compared using a t test. Non-normally distributed (nonparametric) clinicopathologic variables were compared using a Mann-Whitney U test. Platelet count, hematocrit (HCT), and plasma fibrinogen concentration were compared between all CIE dogs vs HC. A t test was performed for platelet count and HCT, and Mann-Whitney U test for plasma fibrinogen concentration.

To assess for correlations between TEG variables and CCECAI, serum albumin, cobalamin, folate, 25(OH)D, plasma fibrinogen, and AT, a Spearman (rank-based) test was performed. For Spearman testing, a statistically significantly correlation score of (±) 0.3 to 0.5 was considered a weak correlation, (±) 0.5 to 0.7 a moderate correlation, and (±) 0.7 to 1.0 a strong correlation.20

After Spearman testing, a Bonferroni correction was performed to account for multiple testing.

Statistical analysis was performed using GraphPad Prism scientific statistic software (Graph Pad Prism, GraphPad Software, Inc, San Diego, California). Significance for all statistical comparisons was set at P < .05 and adjusted after Bonferroni corrections.

### 3 | RESULTS

Twenty-five dogs with CIE were enrolled. Fifteen dogs were castrated males, 1 dog was an intact male, and the remainder were spayed females. Median age of CIE dogs was 6 years (range, 1-11 years). Breeds included mixed breed (8), Bernese mountain dog (4), German shepherd dog (2), and 1 each of American Eskimo, Australian shepherd, Cavalier King Charles spaniel, English bulldog, German shorthaired pointer, Golden retriever, Jack Russell terrier, Labrador retriever, pug, Siberian husky, and Welsh Pembroke corgi. Median body weight was 24 kg (range, 4-47 kg). Sixteen dogs had a serum albumin concentration ≥2.5 g/dL and 9 dogs had serum albumin concentration <2.5 g/dL. Median serum albumin concentration of all CIE dogs was 2.7 g/dL (range, 1.3-3.9 g/dL). Median serum albumin concentration of CIE-PLE dogs (n = 9) was 1.7 g/dL (range, 1.3-1.9 g/dL); median serum albumin concentration of CIE-N dogs (n = 16) was 3.2 g/dL (range, 2.5-3.9 g/dL). Data from 14 healthy control beagle dogs were available for comparisons. Median age of beagle dogs was 1 year (range, 0.5-3 years) and median body weight was 11 kg (range, 9-15 kg). Median serum albumin concentration of beagle dogs was 3.6 g/dL (range, 3.2-3.9 g/dL).

Hemostatic data, including TEG variable data, and results of statistical comparisons for CIE vs HC dogs are shown in Table 1. Notably, CIE dogs had higher MA (P < .001), lower LY30 (P < .001), lower LY60 (P < .001), and longer CLT (P < .001) compared to HC. Plasma fibrinogen concentrations also were higher in dogs with CIE vs HC (P = .007). Dot plots for comparisons of MA, LY60, and fibrinogen between CIE vs HC dogs are shown in Figures 1 to 3.

Both CIE-N and CIE-PLE dogs had higher MA, lower LY30, lower LY60, and longer CLT compared to HC (Table 2). Furthermore, using MA > 60 mm to define hypercoagulability, 19/25 (76%) dogs with CIE were considered hypercoagulable including 9/9 hypoalbuminemic dogs and 10/16 (63%) normoalbuminemic dogs. Platelet count and HCT were not different between CIE vs HC dogs.

Platelet aggregometry data were available for 9 CIE dogs (5/9 hypoalbuminemic, 4/9 normoalbuminemic). Median AUC for saline (AUCsaline) was 12 (range, 0-233), AUC for ADP (AUCADP) was 240 (range, 100-338), and AUC for AA (AUCAA) was 185 (range, 12-264) for CIE dogs. Reference ranges at our institution are as follows: AUCsaline 0 to 33; AUCADP 175 to 294; and AUCAA 131 to 325. Based on these reference ranges, 3/9 CIE dogs had increased spontaneous platelet aggregation in saline and 1 dog had an increased response to ADP.

Median CCECAI score for CIE dogs was 9 (range, 2-19). Median CCECAI scores for CIE-PLE and CIE-N dogs were 11.5 (range, 5-17) and 8 (range, 2-19), respectively. The CIE dogs had a median serum

### TABLE 1 Hemostatic data and results of comparisons for chronic inflammatory enteropathy dogs vs healthy control dogs

| Variable            | All CIE dogs (n = 25), median (range) or mean ± SD | HC dogs (n = 14), median (range) or mean ± SD | P value* |
|---------------------|---------------------------------------------------|---------------------------------------------|----------|
| Fibrinogen (mg/dL) | 272 (146-523)                                     | 196 (140-254)                              | .007     |
| Platelet count (x10^3) | 303 ± 141                                       | 251 ± 55                                   | .19      |
| Hematocrit (%)      | 48 ± 7                                            | 46 ± 5                                     | .21      |
| MA-TF-tPA (mm)      | 68 (50-73)                                       | 47 (37-67)                                 | <.001    |
| R (min)             | 1.3 (0.9-2)                                      | 1.9 (1.3-3.1)                              | <.001    |
| K (min)             | 1.2 (0.8-1.9)                                    | 2.1 (0.8-4.4)                              | <.001    |
| a angle (°)         | 74 (63-79)                                       | 63 (42-79)                                 | <.001    |
| LY30 (%)            | 33 (1-63)                                        | 60 (25-75)                                 | <.001    |
| LY60 (%)            | 61 (5-81)                                        | 80 (33-88)                                 | <.001    |
| CLT (min)           | 61 (11-104)                                      | 30 (17-92)                                 | .008     |

Note: R, measure of time to initial fibrin formation; K represents clot formation time; a angle represents the speed of fibrin cross linking; MA indicative of overall clot strength.

Abbreviations: CIE, chronic inflammatory enteropathy; CLT, clot lysis time; HC, healthy control; LY30, % lysis after 30 minutes; LY60, % lysis after 60 minutes; MA, maximum amplitude; TF, tissue factor; tPA, tissue plasminogen activator.

*P value as assessed by Mann-Whitney U test for nonparametric variables [data presented as median (range)] and t test for parametric variables (data presented as mean ± SD). Significance set at P < .05.
cobalamin concentration of 335 ng/L (range, <150 to >1000 ng/L; reference interval [RI], 251-908 ng/L) and median serum folate concentration of 11.2 μg/L (range, 2.3-38.9 μg/L; RI, 7.7-24.4 μg/L). Median serum 25(OH)D concentration was 117 nmol/L (range, 6-339 nmol/L; RI, 109-423 nmol/L). Median plasma AT concentration was 113% (range, 51%-161%; RI, 104%-162%). Plasma AT concentrations were decreased in 10/25 (40%) dogs with CIE; 9/10 of CIE dogs with decreased antithrombin were hypoalbuminemic.

Correlations between TEG variable data and CCECAI, serum albumin, cobalamin, folate and 25(OH)D, plasma fibrinogen, and AT are shown in Table 3. After correction for multiple testing, significant moderate correlations were observed between MA and serum albumin (rho = −0.53, P = .006), serum 25(OH)D (rho = −0.68, P < .001), plasma fibrinogen (rho = 0.68, P < .001), and plasma AT (rho = −0.61, P = .001; Figures 4-7).

4 | DISCUSSION

In our study, both normoalbuminemic and hypoalbuminemic dogs with CIE were hypercoagulable and hypofibrinolytic based on TEG when compared to HC dogs. These findings indicate that dogs with CIE do not have to be hypoalbuminemic to display a hypercoagulable phenotype on TEG. However, a correlation between serum albumin concentration and MA was observed. Additionally, using a cutoff of MA > 60 mm, 100% of hypoalbuminemic CIE dogs were considered hypercoagulable on TEG compared to 63% of normoalbuminemic dogs with CIE. This observation suggests that a relationship between serum albumin concentration and hypercoagulability as assessed by TEG exists, and that hypercoagulability may be more common in dogs with CIE-PLE when compared to dogs with CIE and normal serum albumin concentration. Regardless, based on our results, clinicians should be aware of a possible hypercoagulable state in both normoalbuminemic and hypoalbuminemic dogs with CIE. Whether normoalbuminemic dogs with CIE are predisposed to TE remains unknown.

Plasma fibrinogen concentrations were higher in dogs with CIE compared to HC, and were moderately positively correlated with MA in dogs with CIE. In healthy conditions, MA is inherently dependent on plasma fibrinogen concentration and function, as well as platelet number, platelet function and, variably, HCT.21,22 It is unknown whether this relationship persists in disease states. Several studies in veterinary medicine have found a relationship between fibrinogen and MA or G, a value calculated from MA and used to define the state of coagulation. These include studies in dogs with chronic hepatopathies,23 congenital portosystemic shunts,24 and acute liver injuries.25 These findings suggest that plasma fibrinogen concentration could serve as a surrogate conventional coagulation test for MA when TEG is unavailable. However, the correlation we found was only moderate, and if using fibrinogen as a surrogate for MA, consideration of other variables known to affect MA would be important. Hyperfibrinogenemia likely reflects the ongoing inflammatory state in dogs with CIE, which is a proposed mechanism for the development of TE

**Figure 1** Dot plot of MA-TF-tPA in dogs with CIE compared to HC. Horizontal bar represents median. Interquartile range also shown. CIE, chronic enteropathy; HC, healthy control; MA, maximum amplitude; TF, tissue factor; tPA, tissue plasminogen activator

**Figure 2** Dot plot of LY60 in dogs with CIE compared to HC. Horizontal bar represents median. Interquartile range also shown. CIE, chronic enteropathy; HC, healthy control; LY60, percent of clot lysis 60 minutes after maximum amplitude is reached

**Figure 3** Dot plot of plasma fibrinogen in dogs with CIE compared to HC. Horizontal bar represents median. Interquartile range also shown. CIE, chronic enteropathy; HC, healthy control
TABLE 2  Thromboelastography variable comparisons between healthy controls vs normoalbuminemic dogs with CIE and healthy controls vs hypoalbuminemic dogs with CIE

| Variable                  | HC (n = 14), median (range) | CIE-N (n = 16), median (range) | P value* | CIE-PLE (n = 9), median (range) | P value* |
|---------------------------|----------------------------|--------------------------------|----------|--------------------------------|----------|
| MA-TF-tPA (mm)            | 47 (37-67)                 | 63 (50-71)                     | <.001    | 67 (63-73)                     | <.001    |
| LY30 (%)                  | 60 (25-75)                 | 39 (9-63)                      | .01      | 29 (1-86)                      | .006     |
| LY60 (%)                  | 80 (53-88)                 | 71 (19-81)                     | .009     | 44 (5-93)                      | .006     |
| CLT (min)                 | 30 (17-92)                 | 54 (28-98)                     | .02      | 63 (11-104)                    | .03      |

Abbreviations: CIE, chronic inflammatory enteropathy; CIE-N, normoalbuminemic dogs with chronic inflammatory enteropathy; CIE-PLE, hypoalbuminemic dogs with chronic inflammatory enteropathy; CLT, clot lysis time; HC, healthy control; LY30, % lysis after 30 minutes; LY60, % lysis after 60 minutes; MA, maximum amplitude; TF, tissue factor; tPA, tissue plasminogen activator.

*P value as assessed by Mann-Whitney U test. Significance set at P < .05.

TABLE 3  Correlations between selected TEG-variables and CCECAI, serum albumin, cobalamin, folate and 25(OH)D, plasma fibrinogen, and antithrombin in 25 dogs with CIE

| Variable                  | CCECAI | Serum cobalamin (ng/L) | Serum folate (ug/L) | Serum albumin (g/dL) | Serum 25(OH)D (nmol/L) | Plasma fibrinogen (mg/dL) | Plasma antithrombin (%) |
|---------------------------|--------|------------------------|---------------------|----------------------|------------------------|--------------------------|--------------------------|
| MA-TF-tPA (mm)            | rho = 0.23 | rho = −0.24 | rho = −0.17 | rho = −0.53 | rho = −0.68 | rho = 0.68 | rho = −0.61 |
|                           | P = 0.27 | P = 0.25             | P = 0.42          | P = 0.06             | P = 0.01              | P = 0.01             | P = 0.01               |
| LY30 (%)                  | rho = −0.25 | rho = −0.12 | rho = 0.12  | rho = 0.51  | rho = 0.37  | rho = −0.51 | rho = 0.43  |
|                           | P = 0.22 | P = 0.58             | P = 0.54          | P = 0.09             | P = 0.08              | P = 0.09             | P = 0.03               |
| LY60 (%)                  | rho = −0.22 | rho = −0.14 | rho = 0.12  | rho = 0.54  | rho = 0.37  | rho = −0.52 | rho = 0.45  |
|                           | P = 0.29 | P = 0.49             | P = 0.57          | P = 0.06             | P = 0.08              | P = 0.07             | P = 0.02               |
| CLT (min)                 | rho = 0.19 | rho = 0.22 | rho = −0.03 | rho = −0.34 | rho = −0.26 | rho = 0.20 | rho = −0.33 |
|                           | P = 0.37 | P = 0.29             | P = 0.89          | P = 0.09             | P = 0.23              | P = 0.35             | P = 0.11               |

Note: P values as assessed by Spearman correlation.

Abbreviations: 25(OH)D, serum 25-hydroxyvitamin D; CCECAI, canine chronic enteropathy clinical activity index; CIE, chronic inflammatory enteropathy; CLT, clot lysis time; HC, healthy control; LY30, % lysis after 30 minutes; LY60, % lysis after 60 minutes; MA, maximum amplitude; TEG, thromboelastography; TF, tissue factor; tPA, tissue plasminogen activator.

FIGURE 4  Scatter plot showing relationship between MA-TF-tPA and serum albumin in dogs with CIE. CIE, chronic inflammatory enteropathy; MA, maximum amplitude; TF, tissue factor; tPA, tissue plasminogen activator

in these patients.1,2  Importantly, the role of hyperfibrinogenemia to predict TE in dogs with CIE is unknown.

Dogs with CIE had longer CLTs and lower LY30 and LY60 than HC dogs, suggesting relative hypofibrinolysis. The fibrinolytic system has been extensively investigated in humans with IBD,26 and an increase in inhibitors (such as thrombin-activatable fibrinolysis inhibitor [TAFI])27 as well as a decrease in fibrinolysis activators (such as tPA)28 have been described. In humans with IBD, overall decreased activity of the system, or hypofibrinolysis, has been observed frequently.3 Anti-tPA antibodies have been described in some human IBD patients, and development of these antibodies has been proposed as a mechanism for the hypofibinolytic and prothrombotic state in humans with IBD.29 The reason for the relative hypofibrinolytic phenotype seen in our study dogs with CIE is unknown. Future studies could evaluate plasma concentrations of fibrinolysis activators or inhibitors (such as TAFI) in dogs with CIE and assess correlation with variables associated with fibrinolysis on tPA TEG.

Platelet count was not different between CIE dogs and HC dogs. Increased platelet counts are common in humans with IBD, and have been described in many studies.4 The increased number of platelets is considered a reaction to the inflammatory process, and has been suggested to play a role in the hypercoagulable state of patients with IBD.4 Independent of their concentration in blood, platelets have been observed to aggregate in vitro in >30% of human IBD patients compared to 0% of healthy controls.30 This finding was thought to be a consequence of inflammation, but similar platelet aggregation has not
been found in other inflammatory diseases, suggesting that it may be a specific characteristic of IBD. Platelet reactivity in humans with IBD may be influenced by high circulating concentrations of von Willebrand factor. Also, platelets in patients with IBD overexpress CD40 ligand, which mediates platelet activation. For these reasons, we collected platelet aggregometry data from a subset of CIE dogs in our study. We elected not to perform statistical comparisons with a healthy control population because of the low number of dogs in our CIE group and the high risk of type II error. Three dogs had evidence of spontaneous aggregation and 1 dog had platelet hyperaggregability to ADP. Therefore, we believe platelet dynamics should be investigated in a larger cohort of dogs with CIE and compared to a healthy control population.

Hyperhomocysteinemia results from low concentrations of folic acid, cobalamin and vitamin B6, and frequently is observed in human patients with IBD, likely because of several mechanisms. Hyperhomocysteinemia is an independent risk factor for venous TE in humans. In dogs, a negative relationship between homocysteine and cobalamin concentrations has been observed, but hypercoagulability has not been evaluated previously in the context of hypocobalaminemia, hypofolatemia, or hyperhomocysteinemia. For the purposes of our study, cobalamin and folate were assessed for correlation to TEG variables. No correlations were observed. Hyperhomocysteinemia may not be expected to play an important role in the development of TE in dogs with PLE because homocysteine is largely bound to albumin in circulation. Therefore normohomocysteinemia or a lower degree of hyperhomocysteinemia may be expected in PLE dogs. However, it still may be important to evaluate for hyperhomocysteinemia in hypercoagulable dogs with CIE, because correction of vitamin deficiencies typically is achievable. Furthermore, hyperhomocysteinemia occurs early in the development of cobalamin deficiency, and it would have been preferable to measure homocysteine in our dogs with CIE because its concentration may be increased before cobalamin concentrations are decreased.

The relationship between vitamin D status and extra-skeletal disease has been widely investigated in human medicine, including its role in cancer, cardiovascular disease, autoimmune disease, infectious disease, and inflammatory disease. Similar studies have been performed in dogs, including in dogs with CIE and PLE. Serum 25(OH)D concentrations were evaluated in relationship to TEG variables in our study, and a moderate correlation between serum 25(OH)D concentration and MA was observed. The clinical relevance of this finding is unknown. However, a link between vitamin D deficiency and TE has been proposed. In cell culture, 1,25(OH)2D has been demonstrated to upregulate thrombomodulin, a transmembrane protein expressed by vascular endothelial cells that plays an important role as a natural anticoagulant. Calcitriol also has been determined to downregulate TF. In addition, vitamin D receptor knockout mice display a phenotype of increased thrombogenic activity. Several clinical studies in humans have highlighted the antithrombotic actions of vitamin D. In a randomized controlled clinical trial, patients with deep vein thrombosis or pulmonary TE receiving vitamin D supplementation required significantly lower doses of warfarin when
compared to those receiving placebo plus warfarin. However, more conclusive evidence is needed before a definitive link can be made. Previous studies in dogs with chronic small intestinal disease have identified an association between decreased serum 25(OH)D concentrations and increases in intestinal inflammation and markers of systemic inflammation. Therefore, the inflammatory state in dogs with CIE may be contributing to the decrease in 25(OH)D concentrations and the increase in MA, prompting the relationship between them.

Plasma antithrombin concentrations were moderately negatively correlated with MA in our dogs with CIE. However, similar to a previous study, several of the dogs determined to be hypercoagulable on TEG had normal plasma antithrombin concentrations. This finding supports the generally accepted hypothesis that the mechanism of hypercoagulability in dogs with chronic small intestinal disease is not simply a consequence of loss of antithrombin, but rather likely to be multifactorial in nature.

Importantly, a hypercoagulable and hypofibrinolytic phenotype on TEG has not been reliably correlated with future TE in veterinary patients. For example, MA was within normal limits in 67% of dogs with immune-mediated hemolytic anemia and pulmonary TE. Furthermore, a retrospective study of 39 dogs found no association between thrombosis identified at necropsy and any TEG parameter. This is a limitation of our study and further illustrates that a hypercoagulable TEG tracing does not with 100% accuracy predict thrombosis. Although we can conclude from our study that CIE dogs display a hypercoagulable and hypofibrinolytic phenotype on TEG, we cannot use this information to predict future risk of thrombosis or need for antithrombotic treatment. Thromboelastography is highly influenced by sample collection and processing, as well as many patient factors. Therefore, TEG results from different institutions and in different patient populations cannot necessarily be extrapolated to all studies. Our study was not intended to determine whether hypercoagulable or hypofibrinolytic changes on TEG predict thrombosis in dogs with CIE, but future studies should examine this possibility. Another limitation of our study is the low number of dogs in the study that had platelet aggregometry performed. However, our purpose was to collect pilot data in CIE dogs. Additionally, healthy beagle controls were not age- and sex-matched to the CIE dogs. Finally, although all 26 dogs in the study had a histologic diagnosis of intestinal inflammation, 3/25 did not have their ileum biopsied. Because pathology can differ among sections of the intestine, we could have missed concurrent disease processes in those dogs.

In conclusion, both normoalbuminemic and hypoalbuminemic dogs with CIE were considered hypercoagulable and hypofibrinolytic on TEG compared to healthy controls. Plasma fibrinogen concentration as measured using the Clauss method may be a suitable surrogate for MA in dogs with CIE when TEG is not available. Platelet dynamics should be studied further in dogs with CIE and PLE. Interestingly, low serum 25(OH)D concentration was moderately correlated with increased MA in our study population, but the clinical relevance of this finding is unknown. Correlation between TEG phenotype and TE is needed in dogs with CIE before therapeutic recommendations can be made based on TEG findings alone.

ACKNOWLEDGMENT
No funding was received for this study.

CONFLICT OF INTEREST DECLARATION
Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
Colorado State University Clinical Review Board approval.

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.

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How to cite this article: Wennogle SA, Olver CS, Shropshire SB. Coagulation status, fibrinolysis, and platelet dynamics in dogs with chronic inflammatory enteropathy. *J Vet Intern Med*. 2021;35:892-901. [https://doi.org/10.1111/jvim.16092](https://doi.org/10.1111/jvim.16092)