Thromboelastography in Dogs with Chronic Hepatopathies

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Background: The coagulation status of dogs with liver disease is difficult to predict using conventional coagulation testing.

Hypothesis/Objectives: To evaluate thromboelastography (TEG) results and associations with conventional coagulation results and indicators of disease severity and prognosis in dogs with chronic hepatopathies (CH).

Animals: Twenty-one client-owned dogs.

Methods: Dogs with CH were prospectively (10 dogs) and retrospectively (11 dogs) enrolled from 2008 to 2014. Kaolin-activated TEG was performed and compared with reference intervals by t-tests or Mann-Whitney tests. Correlation coefficients for TEG results and conventional coagulation and clinicopathologic results were determined. Significance was set at P < 0.05.

Results: Dogs with CH had significant increases in R (5.30 min vs 4.33 min), K (3.77 min vs 2.11 min), and LY30 (4.77% vs 0.68%) and decreased angles (55.3° vs 62.4°). G value defined 9 of 21, 7 of 21, and 5 of 21 dogs as normocoagulable, hypercoagulable, and hypocoagulable, respectively. G and MA were correlated with fibrinogen (r = 0.68, 0.83), prothrombin time (PT; r = −0.51, −0.53), and activated partial thromboplastin time (aPTT; r = −0.50, −0.50). K was correlated with PT (r = 0.75) and protein C activity (r = −0.92). Angle was correlated with aPTT (r = −0.63). Clinical score was correlated with PT (r = 0.60), MA (r = −0.53), and R (r = −0.47). Dogs with hyperfibrinolysis (LY30 > 3.04%; 5 of 21) had significantly higher serum transaminase activities. Dogs with portal hypertension had significantly lower G, MA, and angle and prolonged, K, R, and PT.

Conclusions and Clinical Relevance: Dogs with CH have variable TEG results. Negative prognostic indicators in CH correlate with hypocoagulable parameters on TEG. Hyperfibrinolysis in dogs with CH is associated with high disease activity.

Key words: Coagulation; Fibrinolysis; Hemostasis; Liver.

The liver plays an important and complex role in hemostasis. It is the site of synthesis and clearance of most procoagulant and anticoagulant proteins and regulators of fibrinolysis. In addition, many liver disorders are accompanied by endothelial activation leading to increases in procoagulant von Willebrand factor (vWF) and Factor VIII activity. The net result in human patients with liver disease is a rebalanced hemostatic system. This balance is fragile, and concurrent risk factors such as infection, drug treatment, or use of blood products can tip hemostasis into hypocoagulable or hypercoagulable states leading to bleeding or thrombosis, respectively. This complex but tenuous interaction of procoagulant, anticoagulant, fibrinolytic, and endothelial factors in liver disease makes the clinically important prediction of bleeding and thrombotic risk in individual patients extremely challenging.

Dogs with chronic hepatopathies (CH) classically have been thought to be hypocoagulable based on conventional coagulation tests, which are characterized by prolongations in prothrombin time (PT) and activated partial thromboplastin time (aPTT), thrombocytopenia, and decreases in plasma fibrinogen concentration. All of these parameters suggest the presence of hypocoagulability but spontaneous bleeding in dogs with CH is rare. In fact, portal vein thrombosis has been reported as a complication in dogs with CH, which suggests that some dogs with CH might be hypercoagulable. Thus, dogs, like humans with CH, also may be in a state of rebalanced coagulation.
Conventional coagulation tests such as PT and aPTT are inadequate to describe the coagulation state in patients with liver disease. Thromboelastography (TEG) is a whole blood assay that can evaluate clot formation as a dynamic process, measuring clot time and strength, as well as the kinetics of clot formation and lysis. For many years, TEG has been used in human medicine as a bedside test to evaluate coagulation and to guide factor repletion and fibrinolytic treatment in liver transplant patients. In addition, it recently has been shown in humans that TEG can predict bleeding tendencies and thus serve as an accurate guide to blood product use in patients with cirrhosis.

In veterinary medicine, some studies have evaluated TEG in dogs with liver disease. Dogs with extrahepatic bile duct obstruction and congenital portosystemic shunts have TEG parameters suggesting hypercoagulability. In contrast, TEG parameters in dogs with acute liver disease are compatible with a hypocoagulable or normocoagulable state. Dogs with acute liver disease that progress to synthetic failure develop hyperfibrinolysis. To date, TEG findings in dogs with CH have not been reported. Therefore, the objectives of this study were to describe TEG findings in dogs with CH and to compare the coagulation status as determined by TEG to clinical presentation, clinical pathology, conventional coagulation tests, and known prognostic indicators in CH.

Materials and Methods

Study Population

Twenty-one dogs with CH that had TEG analysis as part of their diagnostic evaluation at the Foster Hospital for Small Animals at the Cummings School of Veterinary Medicine at Tufts University were enrolled. From 2008 to 2010, 10 dogs were enrolled as part of a larger prospective study on the role of TEG in liver disease and another 11 dogs were retrospectively enrolled (2010–2014). Inclusion criteria included a diagnosis of CH made by hepatic biopsy (n = 18) or based on clinical valuables (n = 3). All 3 dogs without hepatic biopsy had increases in serum bilirubin concentration, serum liver enzyme activities (alanine aminotransferase and aspartate aminotransferase), hypoalbuminemia, hypocholesterolemia, and low blood urea nitrogen concentration as well as 1 or more ultrasound abnormality consistent with end-stage liver disease (e.g., ascites, multiple acquired portosystemic shunts, and a small nodular liver with irregular margins). Records for cases retrospectively enrolled in the study were reviewed by a board-certified internist (CRW) to determine whether they met criteria for enrollment. Dogs being treated with medications known to affect coagulation (e.g., corticosteroids, nonsteroidal anti-inflammatory drugs, fish oil supplements, vitamin K, clopidogrel, heparin) or with comorbid diseases based on testing performed by the attending clinician that are known to be associated with coagulation derangements (e.g., hyperadrenocorticism, protein-losing enteropathy, protein-losing nephropathy, immune-mediated hemolytic anemia, infectious enteritis, or neoplasia) were excluded. The Clinical Studies Research Committee of the Cummings School of Veterinary Medicine institutional review board approved the study, and all owners whose dogs were enrolled in the prospective study gave informed consent.

Liver biopsy specimens (n = 18) were obtained by percutaneous ultrasound-guided biopsy or laparoscopy. Chronic hepatitis was diagnosed using World Small Animal Association histological criteria and characterized by the presence of cell death, a mononuclear or mixed inflammatory infiltrate, regeneration, and fibrosis. Hepatic copper concentrations (n = 8) were determined at the Colorado State University Diagnostic Laboratory by atomic absorption analysis and concentrations expressed as micrograms per gram of dry weight liver.

Medical records were reviewed, and relevant historical information, clinical signs, physical examination findings, results of CBC, serum biochemical profile, urinalysis, thoracic radiographs, abdominal ultrasound examination, and hepatic biopsy were recorded. Each dog was retrospectively assigned a clinical score ranging from 1 to 13 as previously reported. This clinical score assigns points based on clinical signs (e.g., icterus, ascites, hepatic encephalopathy, polyuria, polydipsia, anorexia, lethargy, vomiting) and clinical pathologic results (e.g., bilirubin, albumin, and aPTT).

Portal hypertension was diagnosed in 8 dogs based on the presence of abdominal effusion consistent with a non-neoplastic, non-inflammatory pure or modified transudate (n = 8), detection of multiple acquired portosystemic shunts (n = 2), and a small liver with or without irregular margins at surgery or abdominal ultrasound examination (n = 8).

Hemostatic Analysis

All coagulation testing was carried out in the Coagulation Laboratory in the Foster Hospital at the Cummings School. All other clinical pathologic testing was carried out at the Cummings School Clinical Pathology Laboratory. Blood was collected for CBC and serum biochemical profile in 20 dogs, and hemostatic testing (PT, aPTT, platelet count, and TEG analysis) in 21 dogs. In a subset of 9 dogs, quantitative fibrinogen, antithrombin activity (AT), and protein C activity (PC) were determined and 8 dogs had d-dimers activity determined. All dogs had urinalysis performed to check for proteinuria. Whole blood for TEG analysis was drawn by peripheral venipuncture with a Vacutainer blood collection needle into plastic tubes containing 3.2% sodium citrate to obtain a dilution of blood to sodium citrate of 9:1, as previously described and in accordance with recent published consensus standards. After a 30-minute rest period at room temperature, a single operaror performed kaolin-activated TEG. Reference intervals for TEG analysis have been established in the Cummings Coagulation Laboratory (see supplemental information) and were used to define abnormal values in the dogs with CH. In some dogs, an additional sample of citrated plasma was stored at −70°C for analysis of quantitative fibrinogen, AT activity, PC activity, and d-dimers.

The following TEG variables were generated: R (a measure of initial fibrin formation), K (indicative of clot formation time), angle (indicative of the rapidity of fibrin cross linking), MA (indicative of overall clot firmness), and LY30 (expressing % clot lysis during 30 minutes after MA was reached). G value, a mathematical manipulation of MA, was calculated and used to define state of coagulation. Based on TEG analysis and established reference intervals in the Coagulation Laboratory, dogs were labeled as hypercoagulable (G value > 8446 d/s, MA > 64.1 mm and R < 1.81 min), normocoagulable, or hypocoagulable (G value < 3867 d/s, MA < 45.4 mm and R > 6.85 min). Hyperfibrinolysis was defined as LY30 > 3.04%.

Statistical Analysis

Box and whisker plots and tests for skewness and kurtosis were used to evaluate data distribution. Parametric and nonparametric data were expressed as mean and standard deviation or median and range, respectively. Platelet count, white blood cell count, hematocrit (Hct), biochemical data, coagulation parameters, and
TEG parameters in dogs with CH were compared with reference intervals established in control dogs by parametric (Student’s t-test or Welch’s t-test with unequal variances) or nonparametric (Mann-Whitney) tests. Because previous studies have demonstrated associations of hyperbilirubinemia, hypoalbuminemia, portal hypertension (ascites, small liver), and clinical score with survival in dogs with CH, we looked for statistical associations of these factors, as well as disease activity indicators (serum aminotransferase activities)\(^\text{19}\) with TEG parameters and conventional coagulation tests using Pearson’s correlation coefficient after log transformation for continuous data, if necessary or Fisher’s exact test (presence/absence of portal hypertension). Statistical significance was set at \(P < .05\) (2-tailed), and post hoc analysis was adjusted for multiple comparisons by Bonferroni correction (\(P < .0063\)). The following descriptors for \(r\) were defined to characterize correlations: very strong (0.8–1.0), strong (0.6–0.79), moderate (0.4–0.59), weak (0.2–0.39), and very weak (0.00–0.19). Statistical analysis was carried out with computer software.\(^5\)

### Results

Twenty-one dogs were enrolled in the study including the following breeds: Labrador retriever (\(n = 8\)), Doberman pinscher (\(n = 2\)), standard poodle (\(n = 2\)), Golden Retriever (2) and 1 of each of cockapoo, West Highland white terrier, Brittany spaniel, German shepherd dog, Italian greyhound, Newfoundland, and Spinone. There were 11 spayed females and 10 castrated males. The median age and weight were 6.5 years (range, 3.5–14 years) and 21.7 kg (range, 5.5–56.3 kg), respectively. Three dogs had been on phenobarbital that may have contributed to the development of CH, but in all dogs, the drug had been tapered and discontinued at least 2 weeks before TEG analysis and hepatic biopsy. Clinical signs included inappetence (13 of 21), vomiting (12 of 21), polydipsia (7 of 21), lethargy (5 of 21), icterus (5 of 21), diarrhea (4 of 21), melena (3 of 21), weight loss (2 of 21), collapse (2 of 21) and 1 each with behavioral change and abdominal distension. A clinical score previously correlated with survival in Labrador retrievers with CH was applied to the dogs in this study.\(^6\) The median clinical score was 4 (range, 2–7).

All dogs had an abdominal ultrasound examination performed or reviewed by a board-certified radiologist. Six of 21 dogs had abdominal effusion, 8 had microhepatia, and 3 each had hepatomegaly or a mixture of both hepatomegaly and microhepatia within different lobes. Six dogs had a nodular liver and 2 had irregular hepatic margins. Thirteen dogs had thoracic radiographs, which were normal except for pleural effusion in 1 dog.

Liver biopsies were obtained from 18 dogs, 6 at laparoscopy, and 12 percutaneously with a 16-gauge needle by ultrasound guidance. All dogs had a biopsy diagnosis of inflammatory CH with or without fibrosis. The main type of inflammation was mixed (10), lymphocytic (4), neutrophilic (2), or granulomatous (1). Fibrosis was present in 11 of 18 biopsies. Copper quantification was performed in 8 dogs. Median hepatic copper content was 1050 \(\mu\)g/g dry weight (range, 167–2050, reference range <400 \(\mu\)g/g dry weight). Five of the 8 had increased copper content. Eight dogs had aerobic and anaerobic bacterial cultures carried out and all were negative.

Twenty dogs had PT, albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) measured, and 19 had hematocrit (Hct) and aPTT measured. One dog had clinicopathologic testing performed by the referring veterinarian. Dogs with CH had higher serum ALT and AST activity, and increased serum bilirubin concentrations as well as significant decreases in platelet, fibrinogen, AT, and PC compared to reference intervals (Table 1). There was no difference in PT, aPTT, and d-dimers between dogs with CH and reference interval. No dog had proteinuria.

All dogs had TEG analysis performed (Table 2). Overall, CH was accompanied by significant mean increases in K, R, and LY30 and decreases in angle compared to the reference interval (Table 2). The G value was consistent with a normocoagulable state in 9 of 21 (42%) dogs, a hypercoagulable state in 7 of 21 (33%) of dogs, and a hypocoagulable state in 5 of 21 (24%) of dogs.

All dogs that were hypocoagulable had prolongations in K and decreased angles, but only 1 of 5 dogs had prolongation in R. None of the dogs that were hypercoagulable had accompanying changes in K, R, or angle. Five of 21 dogs were hyperfibrinolytic with LY30 values from 5.9 to 42.1%.

The associations between TEG parameters and conventional coagulation variables (Hct, PT, aPTT, fibrinogen, PC, AT, d-dimers), white blood cell count and selected serum variables associated with hepatobiliary synthetic function (albumin, bilirubin) or grade of hepatic injury (ALT, AST, clinical score) were investigated. Significant strong positive correlations were found between K and PC (\(r = 0.75\), \(P = .001\)) and a very strong negative correlation between K and PC (\(r = -0.92\), \(P = .0005\)). There was a strong negative correlation between angle and aPTT (\(r = -0.63\), \(P = .004\)). MA had moderate negative correlations with PT (\(r = -0.51\), \(P = .023\)) and aPTT (\(r = -0.50\), \(P = .042\)) and a strong positive correlation with fibrinogen (\(r = 0.68\), \(P = .043\)). Similar correlations were seen for G (PT, \(r = -0.528\), \(P = .017\); aPTT, \(r = -0.50\), \(P = .025\); fibrinogen, \(r = 0.83\), \(P = .003\)). There were no correlations between K, R, MA, angle and G or MA and serum bilirubin, serum albumin, white blood cell count, or serum transaminase activities. Although platelet count and Hct can affect MA and thus G value in normal dogs,\(^3\) there was no correlation between these parameters and G and MA in dogs with CH.

Clinical score had a moderate negative correlation with G (\(r = -0.53\), \(P = .029\)) and R (\(r = -0.47\), \(P = .049\)) and a strong positive correlation with PT (\(r = 0.60\), \(P = .008\)).

Eight dogs were labeled as having clinical signs of portal hypertension. The K and PT were significantly increased, and angle, MA and G were significantly decreased in dogs with portal hypertension (Table 3).

In the 5 dogs that were hyperfibrinolytic, G values labeled 2 as hypocoagulable, 2 as normocoagulable, and 1 as hypercoagulable.
prolonged PT, and 2 of 5 had prolonged aPTT. All had normal Hct, white blood cell count, and serum albumin concentrations, and 1 dog had mild thrombocytopenia. Among the various clinicopathological variables examined, only serum ALT and AST activity was significantly increased in dogs that were hyperfibrinolytic compared to those that were not hyperfibrinolytic. There was no difference in any of the TEG parameters other than LY30 in dogs that did and did not have hyperfibrinolysis (Table 4).

Discussion

In our study, dogs with CH had complex and variable changes in coagulation status. On conventional coagulation testing, many showed deficiencies in procoagulants (PT, aPTT, fibrinogen, and platelets) that could predispose them to bleeding, as well as decreases in anticoagulants (AT, PC) that could predispose them to thrombosis. Similar variability was evident on TEG analysis where G values in individual dogs were

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference Interval</th>
<th>CH Median (range)</th>
<th>Number Above Reference</th>
<th>Number Below Reference</th>
<th>P-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (seconds)</td>
<td>6.2–9.3</td>
<td>9.25 (7.3–21.4)</td>
<td>8/20</td>
<td>0/20</td>
<td>.88</td>
</tr>
<tr>
<td>aPTT (seconds)</td>
<td>8.9–16.3</td>
<td>14.7 (9.3–25.1)</td>
<td>7/19</td>
<td>0/19</td>
<td>.88</td>
</tr>
<tr>
<td>Platelets (x10⁹/L)</td>
<td>180–525</td>
<td>181 (53–449)</td>
<td>0/21</td>
<td>10/21</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>117–455</td>
<td>162 (76–299)</td>
<td>0/9</td>
<td>3/9</td>
<td>.024</td>
</tr>
<tr>
<td>PC activity (%)</td>
<td>73–85</td>
<td>58.8 (8.8–86.6)</td>
<td>0/8</td>
<td>7/8</td>
<td>.021</td>
</tr>
<tr>
<td>AT activity (%)</td>
<td>89–146</td>
<td>52.0 (18.3–80.9)</td>
<td>0/9</td>
<td>8/9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>D-dimers (ng/mL)</td>
<td>121–547</td>
<td>548 (106–2000)</td>
<td>2/8</td>
<td>0/8</td>
<td>.47</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39–55</td>
<td>40 (20–58)</td>
<td>1/19</td>
<td>7/19</td>
<td>.66</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.8–4.0</td>
<td>3.1 (1.6–4.1)</td>
<td>0/20</td>
<td>6/20</td>
<td>.46</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.1–0.3</td>
<td>0.8 (0.1–18.9)</td>
<td>16/20</td>
<td>0/20</td>
<td>.003</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>14–86</td>
<td>441 (45–3005)</td>
<td>18/20</td>
<td>0/20</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Table 2. TEG parameters in 21 dogs with chronic hepatopathies (CH).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Reference Interval Mean ± SDa</th>
<th>CH Mean ± SD</th>
<th>Number Above Reference</th>
<th>Number Below Reference</th>
<th>P-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (min)</td>
<td>4.33 ± 1.26</td>
<td>5.3 ± 2.04</td>
<td>3/21</td>
<td>0/21</td>
<td>.029</td>
</tr>
<tr>
<td>K (min)</td>
<td>2.11 ± 0.69</td>
<td>3.77 ± 3.25</td>
<td>5/21</td>
<td>0/21</td>
<td>.021</td>
</tr>
<tr>
<td>Angle (°)</td>
<td>62.4 ± 7.13</td>
<td>55.3 ± 14.3</td>
<td>0/21</td>
<td>6/21</td>
<td>.023</td>
</tr>
<tr>
<td>Maximum Amplitude (mm)</td>
<td>54.7 ± 4.68</td>
<td>53.1 ± 13.4</td>
<td>5/21</td>
<td>5/21</td>
<td>.45</td>
</tr>
<tr>
<td>G (d/s)</td>
<td>6.16 ± 1.14</td>
<td>6.55 ± 3.53</td>
<td>7/21</td>
<td>5/21</td>
<td>.31</td>
</tr>
<tr>
<td>LY30 (%)</td>
<td>0.68 ± 1.18</td>
<td>4.77 ± 10</td>
<td>5/21</td>
<td>0/21</td>
<td>.046</td>
</tr>
</tbody>
</table>

Table 3. Hemostatic parameters in dogs with chronic hepatopathies with and without portal hypertension.

<table>
<thead>
<tr>
<th>Hemostatic Variable</th>
<th>Reference Intervala</th>
<th>Without Portal Hypertension n = 13</th>
<th>With Portal Hypertension n = 8</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (min)</td>
<td>2.11 ± 0.69</td>
<td>2.5 ± 1.7</td>
<td>5.5 ± 4.4</td>
<td>.036</td>
</tr>
<tr>
<td>R (min)</td>
<td>4.33 ± 1.26</td>
<td>5.4 ± 2.3</td>
<td>5.1 ± 1.5</td>
<td>.76</td>
</tr>
<tr>
<td>Angle (°)</td>
<td>62.4 ± 7.13</td>
<td>60.7 ± 13.1</td>
<td>46.6 ± 11.7</td>
<td>.038</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>54.7 ± 4.68</td>
<td>57.7 ± 12.2</td>
<td>45.3 ± 13.0</td>
<td>.024</td>
</tr>
<tr>
<td>LY 30 (%)</td>
<td>0.68 ± 1.18</td>
<td>2.2 ± 5.8</td>
<td>8.6 ± 14.8</td>
<td>.081</td>
</tr>
<tr>
<td>G (dynes/s)</td>
<td>6.16 ± 1.14</td>
<td>7.75 ± 3.68</td>
<td>4.58 ± 2.46</td>
<td>.042</td>
</tr>
<tr>
<td>aPTT (seconds)</td>
<td>8.9–16.3</td>
<td>15.5 ± 4.2</td>
<td>15.5 ± 4.2</td>
<td>.64</td>
</tr>
<tr>
<td>PT (seconds)</td>
<td>6.2–9.3</td>
<td>9.4 ± 2.03</td>
<td>12.5 ± 4.8</td>
<td>.018</td>
</tr>
<tr>
<td>Platelet (x10⁹/L)</td>
<td>180–525</td>
<td>184 ± 112</td>
<td>175 ± 66</td>
<td>.75</td>
</tr>
</tbody>
</table>

aValues are shown as plus and minus standard deviation or as a reference range.

bValues generated with either Welch’s t-test for unequal variances (TEG parameters) or Student’s t-test for equal variances (aPTT, PT, and platelet count). Significance set at P < .05.
consistent with a hypocoagulable, hypercoagulable, or normocoagulable state in 24%, 33%, and 43% of dogs, respectively. Hypocoagulable variables on TEG (prolonged R and K values, decreased angle, increases in LY30) were associated with abnormalities seen in late stage CH such as decreased plasma fibrinogen concentration, prolonged PT and aPTT, high clinical scores, and the presence of portal hypertension. Further complicating the coagulation picture in dogs with CH was the finding that 25% of the dogs were hyperfibrinolytic. This hyperfibrinolysis, which also would contribute to the hypocoagulable state, was associated with a higher grade of disease as reflected by high serum transaminase activities. Collectively, the results indicate that, as in humans, dogs with CH have variable coagulation alterations associated with a rebalanced state of hemostasis reflecting loss of procoagulants, anticoagulants, and fibrinolytic factors.1,17

Many of the dogs with CH (43%), as do human patients with CH or cirrhosis, maintained overall normal global hemostasis as assessed by TEG. In human cirrhotics, MA does however tend to decrease in proportion to the severity of liver disease,18 suggesting that hypocoagulable tendencies may predominate with end-stage hepatic disease. In our study, TEG parameters predictive of a hypocoagulable state (i.e, decreased MA, G, and angle and increases in K) as well as prolonged PT were more common in dogs with signs of portal hypertension, a complication known to accompany end-stage CH in dogs.128 In addition, MA and angle were negatively correlated with worsening of the clinical score. These results suggest that, as in humans, overall clot strength in dogs with CH may decrease in later stages of liver disease.

Our study identified the presence of hyperfibrinolysis in 5 of 21 dogs (24%) with CH. Previous studies by TEG analysis have shown that hyperfibrinolysis accompanies acute liver failure, trauma, and disseminated intravascular coagulation in dogs.21,33–36 In dogs with acute liver injury, increased LY30 correlates with high white blood cell count and hepatic synthetic failure (e.g, prolonged PT, aPTT, hypercholesterolemia, decreased PC).23 In dogs with CH, LY30 did not correlate with white blood cell count, PT, aPTT, cholesterol, or PC, but was associated with high serum ALT and AST activity, suggesting that hyperfibrinolysis in dogs with CH may be associated with the grade of liver injury (e.g, necrosis, inflammation, degeneration).30 Additional studies investigating a larger number of dogs with CH and hyperfibrinolysis with careful consideration of histological grade and stage of hepatic biopsy samples as well as characterization of the inflammatory cytokine milieu in these patients are needed.

In humans, hyperfibrinolysis can be associated with bleeding from mucosal surfaces, particularly gastrointestinal hemorrhage.37,39 It is currently unknown whether hyperfibrinolysis in dogs with liver disease is associated with bleeding tendencies. A previous study indicated that dogs with acute liver failure, a population with a high prevalence of hyperfibrinolysis, have a high prevalence of bleeding complications.40 It was not possible to discern whether hyperfibrinolytic dogs in our study had bleeding tendencies because they either did not undergo provocative procedures or were preemptively treated with fresh frozen plasma, protease inhibitors, or both before such procedures.

It currently is unknown whether hyperfibrinolysis in dogs with CH is primary or secondary. Secondary hyperfibrinolysis, which occurs in disseminated intravascular coagulation and in trauma associated coagulopathy, is associated with activation of the coagulation system, and is marked by an increase in d-dimers.41 D-dimers are specific plasmin-mediated breakdown products of cross-linked fibrin and are increased only when there is activation of thrombin to form cross-linked fibrin and secondary fibrinolysis. In primary fibrinolysis, which occurs with liver disease and neoplasia in humans, there is no activation of thrombin and thus no increase in d-dimers.41 None of the hyperfibrinolytic dogs in this study had d-dimer concentrations shown), and lack of clinical evidence of thrombosis in any of the dogs with increased LY30 in this study suggests that secondary hyperfibrinolysis was not present. Determining whether hyperfibrinolysis is primary or secondary is important, because clinical bleeding in primary hyperfibrinolysis responds better to treatment
with antiproteases. The use of protease treatment to treat hyperfibrinolysis in human patients undergoing liver transplantation has been instrumental in decreasing the use of blood products during surgery. Hyperfibrinolysis in humans is associated with increases in tissue-type plasminogen activator concentrations and a decrease in fibrinolysis inhibitors such as thrombin-activatable fibrinolytic inhibitor, antiplasmin, and plasminogen activator inhibitor. Evaluation of the contributing factors of fibrinolytic factors will be necessary for a full understanding of the hyperfibrinolytic state in dogs with liver disease.

Because TEG analysis is a relatively insensitive method for detection of accelerated fibrinolysis in humans, the incidence of hyperfibrinolysis could be even higher in dogs with CH. Recent studies in humans and dogs have shown that ex vivo addition of tissue plasminogen activator (TPA) to the TEG assay results in a more sensitive test for increased fibrinolysis. Studies evaluating TPA-TEG in dogs with liver disease currently are underway at Cummings.

Discordance between conventional coagulation tests currently used to assess bleeding risk in dogs with CH and TEG analysis was evident in our study. Using G, we found 76% (16 of 21) of dogs with CH to be normal (43%, 9 of 21) or hypercoagulable (33%, 7 of 21). Despite this, 90% (14 of 16) of these normocoagulable or hypercoagulable dogs had prolongations in PT or aPTT or platelet count on TEG or aPTT. Factor VIII activity secondary to activation of the endothelium by portal hypertension, decreased clearance of intestinal derived endotoxin by the liver or both could have contributed to procoagulant activity. In human patients, loss of procoagulants in cirrhosis also is balanced by increased concentrations of vWF and Factor VIII activity. Activation of the endothelium by portal hypertension, decreased clearance of intestinally derived endotoxin by the liver or both. Neither vWF nor Factor VIII were measured in our study, but should be the subject of future studies in dogs with CH.

Whether TEG is a better predictor of bleeding tendencies than PT, aPTT, and platelet count in dogs with CH is unknown. Although PT and aPTT are known to be poor predictors of bleeding in humans with CH, a relationship between fibrinogen concentration and function, platelet number and function and Hct. These relationships might or might not be maintained in disease states. In our study, G and MA had strong positive correlations with fibrinogen concentration, but not platelet count or Hct. In separate studies ex vivo addition of tissue plasminogen activator (TPA) to PT-TEG in dogs with CH was associated with prolongations in G, M value. In health, MA (and thus G) is intrinsically dependent on fibrinogen concentration and function, platelet number and function and Hct. These relationships might or might not be maintained in disease states. In our study, G and MA had strong positive correlations with fibrinogen concentration, but not platelet count or Hct. In several reports of dogs with CH, aPTT or platelet count were strongly positively correlated with G values in dogs with congenital portosystemic shunts and acute liver injury. In addition, dogs with extrahepatic bile duct obstruction have both high fibrinogen concentrations and low MA. Neither vWF nor Factor VIII were measured in our study, but should be the subject of future studies in dogs with CH.

If dogs with CH, like people, are in a fragile state of rebalanced state of hemostasis, it will be important to determine what factors tip coagulation in favor of bleeding or thrombosis. In humans, sepsis, systemic inflammatory response syndrome, anemia, surgery and the presence of ascites can trigger a hypercoagulable state, whereas concurrent pancreatitis, uncontrolled hepatic encephalopathy, anesthesia, altered portal blood flow dynamics, corticosteroid use, bacterial translocation, and transfusion of blood products can provoke a hypercoagulable state. It will be important to define similar risk factors in dogs with different forms of liver disease. Some evidence exists that altered blood flow, hepatic encephalopathy, and pancreatitis may be risk factors for a hypercoagulable state in dogs with congenital portosystemic shunts and that concurrent
corticosteroid use may be a risk factor for a hypercoagulable state in dogs with CH.\textsuperscript{13}

Our study has several limitations. Conducted as a pilot study to evaluate TEG in dogs with CH, it was designed to be mostly descriptive and thus may have been underpowered to detect significant correlations. In our study, kaolin-activated TEG was performed; it is not known whether a different activator such as tissue factor would yield different results. This possibility should be the subject of future studies. Not all of the dogs in our study had histological confirmation of CH, although the 3 dogs that did not have a biopsy performed had serum biochemical and imaging results consistent with end-stage liver disease. Because only some of the dogs had additional conventional coagulation testing beyond PT, aPTT, and platelet count, it was difficult to draw conclusions about the origin of the state of coagulation. Our study also was not designed to determine whether hypocoagulable or hypercoagulable changes in TEG were correlated with bleeding or clotting tendencies, respectively.

Conclusion

In conclusion, our study indicates that dogs with CH can be either hypercoagulable or hypocoagulable on TEG analysis. A hypocoagulable state may be more common in advanced disease when portal hypertension is present. Fibrinogen had a strong positive correlation with G value, and thus, the value of fibrinogen concentration in predicting the state of coagulation in dogs with CH should be further investigated. Lastly, future studies should be aimed at determining the role of TEG analysis in predicting bleeding and clotting tendencies in dogs with CH.

Footnotes

\textsuperscript{a} ACL Elite Analyzer, Beckman Coulter, Brea, CA
\textsuperscript{b} TEG 5000 Thromboelastograph, Haemonetics Corp, Braintree, MA
\textsuperscript{c} SAS statistical software, version 9.3, SAS Institute A/S, Cary, NC
\textsuperscript{d} Wennogle S, Bradley A, Olver C, Twedt S. Measure of plasma fibrinogen in dogs with hepatobiliary disease. J Vet Intern Med 2015;1195A

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\textbf{Off-label Antimicrobial Declaration:} Authors declare no off-label use of antimicrobials.

References


**Supporting Information**

Additional Supporting Information may be found online in the supporting information tab for this article:

**Data S1.**