

## Thromboelastographic Evaluation of Dogs with Acute Liver Disease

D. Kelley, C. Lester, S. Shaw, A. deLaforcade, and C.R.L. Webster

**Background:** Given the liver's pivotal role in hemostasis and fibrinolysis, the coagulopathy accompanying hepatic disease is complex.

**Hypothesis/Objectives:** To prospectively evaluate kaolin-activated thromboelastography (TEG) in dogs with acute liver disease (ALD) and compare with plasma-based coagulation tests.

**Animals:** Twenty-one dogs with a diagnosis of ALD based on recent onset of clinical signs accompanied by increases in serum bilirubin concentration and alanine aminotransferase activity.

**Methods:** Clinical presentation, CBC, serum biochemistry, platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), and TEG analysis were evaluated in 21 dogs with a subset also having fibrinogen, antithrombin (AT) activity, protein C (PC) activity, d-dimers, and von Willebrand's factor (vWF) activity analyzed. A PT >1.5 times the upper limit of normal defined acute liver failure (ALF).

**Results:** Dogs with ALD had mean increases in R, K, LY30, PT, aPTT, and vWF activity, and decreases in angle, maximal amplitude (MA), G, AT activity, and PC activity. The TEG results defined dogs as hypocoagulable (11/21), normocoagulable (8/21), or hypercoagulable (2/21). Increases in LY30 defined 8/21 dogs as hyperfibrinolytic. Hypocoagulable and hyperfibrinolytic dogs had lower fibrinogen and PC activity than dogs without these abnormalities. Overall, ALF dogs had greater increases in K and LY30, and decreases in MA, G, and PC activity than dogs with less severe hepatic impairment. Results for MA and LY30 were positively correlated with serum bilirubin concentration and white blood cell count, and negatively correlated with serum cholesterol concentration.

**Conclusions and Clinical Importance:** ALD dogs have a range of coagulation abnormalities that trend toward hypocoagulability and hyperfibrinolysis as functional impairment occurs.

**Key words:** bilirubin; coagulation; fibrinolysis; hepatic.

The liver plays a central role in hemostasis as the site of synthesis, clearance or both of most procoagulants, anticoagulants, and regulators of fibrinolysis. Classically, dogs with ALD, many of which have prolongations in PT and aPTT on conventional plasma-based coagulation testing, were thought to be at risk of bleeding from invasive procedures, such as hepatic biopsy.<sup>1–4</sup> In humans with ALD however prolongations in PT and aPTT, unless marked, are not considered accurate predictors of bleeding tendencies.<sup>5–8</sup> Instead, patients with ALD are thought to have a rebalanced coagulation axis with decreased synthesis of procoagulant factors being balanced by loss of anticoagulants. This new balance however is precarious, and coagulation can be shifted toward bleeding or thrombosis by comorbidities such as infection, systemic inflammatory response syndrome (SIRS), or neoplasia.<sup>5–7</sup>

It is crucial to accurately identify the state of coagulation in dogs with ALD. Often, an invasive procedure

### Abbreviations:

ALD	acute liver disease
ALF	acute liver failure
aPTT	activated partial thromboplastin time
AT	antithrombin
DIC	disseminated intravascular coagulation
MA	maximal amplitude
PC	protein C
PT	prothrombin time
SIRS	systemic inflammatory response syndrome
TEG	thromboelastography
tPA	tissue plasminogen activator
uPA	urokinase-like plasminogen activator
vWF	von Willebrand's Factor

such as a liver aspirate or biopsy is necessary for definitive diagnosis so that appropriate treatment can be initiated. Using conventional coagulation tests to guide decisions may overestimate bleeding risk in early ALD precluding acquisition of an aspirate or biopsy. More importantly, relying on PT and aPTT can lead to the administration of costly and potentially harmful transfusion products, such as fresh frozen plasma, when these products are not indicated.<sup>8</sup>

Thromboelastography is a whole blood assay that provides information about the speed of clot formation, clot strength, and clot lysis, and might more accurately predict the hemostatic state in ALD compared to conventional plasma-based coagulation tests. Clinically, TEG has been used to guide transfusion requirements during liver transplantation in people in whom its use decreases blood product requirement and increases patient survival.<sup>6</sup> In humans with cirrhosis, TEG analysis is more reliable than PT in predicting gastrointesti-

From the Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA (Kelley, deLaforcade, Webster); Ocean State Veterinary Specialists, East Greenwich, RI; and VCA Animal Hospitals, Los Angeles, CA.

Corresponding author: Dr. Cynthia R.L. Webster, 200 Westboro Road, North Grafton, MA 01536; e-mail: [cynthia.leville-webster@tufts.edu](mailto:cynthia.leville-webster@tufts.edu).

Submitted December 2, 2014; Revised May 1, 2015; Accepted May 7, 2015.

Copyright © 2015 The Authors. Journal of Veterinary Internal Medicine published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

DOI: 10.1111/jvim.13441

nal rebleeding and sepsis-induced hypocoagulability.<sup>9,10</sup> Initial studies in humans with ALD or chronic cholestasis found that TEG tracings were more compatible with normal coagulation or hypercoagulability than hypocoagulability.<sup>7,11</sup>

Previous studies in dogs with ALD have documented changes in primary and secondary hemostasis. Alterations in primary hemostasis include both thrombocytopenia and thrombocytopathia, whereas changes in secondary hemostasis include decreased activity of procoagulant factors (factors I, II, V, VII-XIII), anticoagulants, AT, and PC.<sup>1-4,12</sup> In veterinary medicine, TEG has been evaluated in a limited number of dogs with liver disease. Similar to humans with cholestasis, dogs with extrahepatic bile duct obstruction most often are hypercoagulable.<sup>13</sup> In addition, dogs with portosystemic shunts appear to be mildly hypercoagulable.<sup>14</sup> No studies to date have reported TEG findings in dogs with ALD.

The objectives of this study were to describe TEG findings in dogs with ALD and to compare their coagulation status as determined by TEG to clinical presentation, serum biochemistry, and conventional coagulation tests.

## Materials and Methods

### Study Population

Twenty-one dogs with ALD were prospectively enrolled. Inclusion criteria included an acute onset of clinical signs (<1 month) associated with increased serum bilirubin concentration and increased serum alanine aminotransferase activity (>3 times the upper limit of normal). Dogs on medications known to affect coagulation (eg, corticosteroids, nonsteroidal anti-inflammatory drugs, fish oil supplements, vitamin K, antiplatelet drugs, anticoagulants) or with comorbidities associated with coagulation derangements (eg, hyperadrenocorticism,<sup>15</sup> protein-losing enteropathies,<sup>16</sup> protein-losing nephropathy,<sup>17</sup> immune-mediated hemolytic anemia,<sup>18</sup> immune-mediated thrombocytopenia,<sup>19</sup> infectious enteritis,<sup>20</sup> or extrahepatic neoplasia<sup>21</sup>) were excluded. Additionally, purebred Greyhounds were excluded because of known alterations in clotting kinetics and clot formation.<sup>22</sup>

Dogs were stratified by severity of hepatic impairment and labeled as having acute liver failure (ALF) when the PT was >1.5 times the upper limit of normal. Because there currently is no standard for defining ALF in dogs, we used criteria developed in humans in whom the most widely accepted definition of ALF includes evidence of a coagulation abnormality, usually an international normalized ratio >1.5 times the upper limit of normal in a patient without preexisting liver disease and a short duration of illness.<sup>8,23</sup> In adults, any degree of alteration in mental status (encephalopathy) also is used to define ALF, although in children encephalopathy is variably present. Because dogs present some of the same challenges in defining encephalopathy as in children, we chose not to include this criterion to define ALF.

### Hemostatic Analysis

At the time of admission, blood was collected for CBC, biochemistry profile, TEG, platelet count, and hemostatic testing (PT, aPTT, quantitative fibrinogen, AT activity, PC activity, d-dimers, and vWF activity). Whole blood for TEG analysis was drawn by peripheral venipuncture with a vacutainer blood collection needle into tubes containing 3.2% sodium citrate to obtain a dilution of blood-to-sodium citrate of 9 : 1. Additional blood was drawn into

an EDTA tube for a CBC. After a 30-minute hold period at room temperature, a single operator performed kaolin-activated TEG. Reference ranges for TEG variables were established in the Coagulation Laboratory in the Foster Hospital at the Cummings School.<sup>14</sup> The remaining citrated plasma was stored at  $-80^{\circ}\text{C}$  for analysis of PT,<sup>a,b</sup> aPTT,<sup>a,b</sup> quantitative fibrinogen,<sup>a,b</sup> AT activity,<sup>a,b</sup> PC activity,<sup>a,b</sup> d-dimers,<sup>a,b</sup> and vWF activity.<sup>a,b</sup> All coagulation testing was conducted in the Coagulation Laboratory at Cummings. The CBC and serum biochemistry panels were performed in the Cummings School clinical pathology laboratory. The Clinical Studies Research Committee, the Cummings School of Veterinary Medicine institutional review board, approved the study and all owners gave written consent.

The following TEG variables were recorded: *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 minute after *MA* was reached). The *G* value, a mathematical manipulation of *MA*, was calculated.<sup>24</sup> Depending on TEG analysis, dogs were labeled as hypercoagulable (*G* value > 8446 d/s, *MA* > 64.1 mm, *R* < 1.81 minute or some combination of these), normocoagulable, or hypocoagulable (*G* value < 3867 d/s, *MA* < 45.4 mm, *R* > 6.85 minute or some combination of these).

### 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, hematocrit, WBC count, biochemical data, coagulation parameters, and TEG variables in dogs with liver disease were compared with reference ranges using parametric (Student's *t*-test) or nonparametric (Mann-Whitney) tests. Correlations between conventional plasma tests or serum biochemical variables and TEG variables were done with Pearson's correlation coefficient. In some cases, nonparametric data were log transformed. Statistical significance was set at  $P < .05$  (2-tailed) and adjusted for multiple comparisons by Bonferroni correction.

## Results

The 21 dogs enrolled in the study consisted of 3 mixed breed dogs and 18 pure bred dogs including Bernese mountain dog ( $n = 2$ ), pug ( $n = 2$ ), dachshund ( $n = 2$ ), standard poodle ( $n = 2$ ), German shepherd dog ( $n = 2$ ), and 1 each of golden retriever, Australian shepherd, border collie, Newfoundland, Belgium shepherd, dalmatian, bearded collie, and soft-coated wheaten terrier. There were 11 spayed females, 3 intact males, and 7 castrated males. The median age and weight were 5 years (range, 0.7–11 years) and 19 kg (range, 5.4–66.8 kg), respectively. Clinical signs included lethargy (14/21), inappetence (14/21), vomiting (11/22), diarrhea (6/21), and 1 each with hematochezia, polydipsia and polyuria, ptialism, ataxia, and ascites. The cause of ALD in the 21 dogs was determined by review of the medical record (CRLW, CL) and included idiopathic (11/21), neoplasia (4/21), drug toxicity (2/21), immune-mediated (2/21), and infectious (2/21). Of the 21 dogs, 6 dogs did not survive to discharge; 2/6 had neoplasia and 4/6 were considered idiopathic. All dogs had abdominal ultrasound examination performed with no signs of biliary disease or bile duct obstruction.

None had focal hepatic lesions. Seven of 21 dogs had detectable abdominal effusion on ultrasound examination.

All 21 dogs had TEG analysis performed. Overall, dogs with ALD had significant mean decreases in angle, MA, and G, and significant increases in R and LY30 compared to reference ranges (Table 1). The G value labeled 11/21 dogs as hypocoagulable, 8/21 as normocoagulable, and 2/21 as hypercoagulable. Eight dogs were labeled as hyperfibrinolytic with LY30 values from 7.9 to 59%.

The PT, aPTT, and platelet count were performed in all dogs, AT activity, PC activity, and d-dimers in 11 dogs, fibrinogen in 10 dogs, and vWF activity in 8 dogs. Dogs with ALD had significant decreases in platelet count and AT activity, and significant increases in PT, aPTT, and vWF activity compared to reference ranges (Table 2). Median platelet count (automated with manual smear evaluation) was lower than the reference value in dogs with ALD, but still within the reference range. Eight of 21 dogs had thrombocytopenia ranging from 13,000/ $\mu$ L to 174,000/ $\mu$ L (median, 87,000/ $\mu$ L). There was no difference for d-dimers, PC activity, and fibrinogen results between dogs with ALD and reference ranges.

A CBC and serum biochemistry panel were performed in all dogs. The median hematocrit was within the reference range (median, 44%; range, 20–63%), but 8/21 dogs were anemic (median, 32.5%; range, 20–34%). Six of 21 dogs with ALD had increases in the WBC count (median,  $24.2 \times 10^3$ / $\mu$ L; range,  $17.4$ – $34.2 \times 10^3$ / $\mu$ L). All dogs had increased serum bilirubin concentration (median, 4.5 mg/dL; range, 0.6–49 mg/dL), alanine aminotransferase activity (median, 810 U/L; range, 102–26,670 U/L), and aspartate aminotransferase activity (median, 279 U/L; range, 64–12,306 U/L). Eight of 21 dogs were hypoalbuminemic, 4/21 were hypoglycemic, and 3/17 were hypercholesterolemic.

Coagulation status as determined by TEG analysis was compared to conventional coagulation tests. In the 8 dogs classified as normocoagulable, 4/8 had increases in PT (up to 12 times the upper limit of normal), aPTT (up to 1.78 times the upper limit of normal), or both. Normocoagulable dogs had normal median platelet counts, fibrinogen, d-dimers, PC activity, and vWF

activity with a decrease in AT activity (median, 42%; range, 34–86%). In the hypocoagulable group, 9/10 and 7/8 had prolongations of PT and aPTT, respectively, that were on average 4–5 times the upper limit of normal and significantly longer than the prolongations in normocoagulable dogs. One hypocoagulable dog had a normal PT and aPTT. Hypocoagulable dogs had lower PC activity (median, 32%; range, 15–47%) and were more likely to have a fibrinogen concentration <150 ng/dL than normo- or hypercoagulable dogs. All of the ancillary coagulation testing in the 2 hypercoagulable dogs was normal, but the small number ( $n = 2$ ) precluded statistical comparison. The PCVs of the 2 hypercoagulable dogs were 49 and 33%. In the latter dog, the mild anemia may have contributed to the increase in G.<sup>25</sup>

When dogs were stratified by severity of disease using PT prolongation >1.5 times the upper limit of normal to define ALF, 10 dogs had ALF. These dogs had significantly longer K times, LY30, PT, and aPTT, and significant decreases in angle, MA, G, fibrinogen, and PC activity compared to dogs with less severe ALD or reference values (Figs 1, 2). These dogs also had higher WBC counts and lower serum cholesterol concentration compared to dogs with ALD ( $14.9 \times 10^3$ / $\mu$ L [range,  $6$ – $34.2 \times 10^3$ / $\mu$ L] versus  $10.5 \times 10^3$ / $\mu$ L [range,  $6.7$ – $21.2 \times 10^3$ / $\mu$ L] and 134 mg/dL [range, 86–219 mg/dL] and 295 mg/dL [range, 90–734 mg/dL], respectively). There were no other differences in biochemical variables between dogs with ALD and those with ALF (data not shown).

Eight dogs were hyperfibrinolytic with LY30 values greater than the upper limit of the reference range. Hyperfibrinolytic dogs were more likely to be hypocoagulable (7/8) and have ALF (6/8) than nonhyperfibrinolytic dogs ( $P = .024$  and  $P = .028$ , respectively). Hyperfibrinolytic dogs had significant decreases in angle, MA, and G on TEG, a greater decrease in fibrinogen and PC activity (Figs 3, 4), and higher WBC counts (median,  $16.0 \times 10^3$ / $\mu$ L; range,  $8.6$ – $34.2 \times 10^3$ / $\mu$ L versus median,  $10.8 \times 10^3$ / $\mu$ L; range,  $6$ – $26.1 \times 10^3$ / $\mu$ L) than nonhyperfibrinolytic dogs. The mean platelet count in hyperfibrinolytic dogs ( $222 \times 10^3$ / $\mu$ L; range,  $107$ – $306 \times 10^3$ / $\mu$ L) was similar to that seen in dogs without hyperfibrinolysis ( $174 \times 10^3$ / $\mu$ L; range,  $45$ – $450 \times 10^3$ / $\mu$ L). Only 2 hyperfibrinolytic dogs had platelet counts below the reference

**Table 1.** TEG variables in dogs with acute liver disease.

Variables	ALD Mean $\pm$ SD	Reference Range Mean $\pm$ SD	Number Above Reference	Number Below Reference	<i>P</i> - value <sup>a</sup>
R (min)	5.0 $\pm$ 2.1	4.33 $\pm$ 1.26	4	0	.003
K (min)	5.1 $\pm$ 5.3	2.11 $\pm$ 0.69	8	0	.092
Maximum amplitude (mm)	42.1 $\pm$ 18.5	54.7 $\pm$ 4.7	2	11	.00001
Angle (°)	46.5 $\pm$ 17.6	62.4 $\pm$ 7.1	0	10	.0001
G (d/s)	4.6 $\pm$ 3.1	6.16 $\pm$ 1.14	2	11	.0007
LY30 (%)	14.5 $\pm$ 22.4	0.68 $\pm$ 1.18	8	0	.008

<sup>a</sup>*P*-value for comparison of TEG variables between dogs with acute liver disease and reference range using Student's *t*-test. Bonferroni adjusted *P* < .009.

**Table 2.** Hemostatic testing in dogs with acute liver disease.

Variable	ALD Median (Range)	Reference Median (Range)	Number Above Reference	Number Below Reference	<i>P</i> - value <sup>a</sup>
PT (s)	12.2 (7.1–60)	7.6 (6.2–9.3)	13	0	.001
aPTT (s)	17.9 (12.1–161)	14.8 (8.9–16.3)	11	0	.001
Platelets ( $\times 10^9/L$ )	190 (13–450)	353 (180–525)	0	8	.001
Fibrinogen (mg/ dL)	187 (78.4–620)	232 (73.4–455)	1	0	.53
PC activity (%)	46.5 (15.2–112)	78 (73–85)	0	5	.029
AT activity (%)	71.1 (25–95)	120 (89–146)	0	9	.0001
vWF activity (%)	101 (55.7–145)	65 (73–109)	4	1	.0001
D-dimers (ng/mL)	219 (54.8–471)	251 (121–547)	0	1	.3219

<sup>a</sup>*P*-value for comparison of hemostatic variables in dogs with acute liver disease and reference range using Mann–Whitney test. Bonferroni adjusted *P* < .006.

range ( $107 \times 10^3/\mu L$  and  $110 \times 10^3/\mu L$ ). Four of 8 of the hyperfibrinolytic dogs had systemic inflammatory response syndrome (SIRS), but this percentage was not different than the number of dogs with a SIRS diagnosis (4/13) in the nonhyperfibrinolytic group.<sup>26</sup> Three dogs had bacterial cultures submitted and none were positive. One hyperfibrinolytic dog with a normal platelet count had evidence of gastrointestinal bleeding that resolved with aminocaproic acid treatment. One other dog had spontaneous bleeding from a catheter site and this dog was hypocoagulable on TEG, but not hyperfibrinolytic.

Serum laboratory test results indicative of ALD were compared with TEG parameters. The degree of hyperfibrinolysis (high LY30) positively correlated with PT ( $r = 0.830$ ,  $P < .0001$ ), aPTT ( $r = 0.7631$ ,  $P < .0001$ ), bilirubin ( $r = 0.764$ ,  $P = .0003$ ) and WBC count ( $r = 0.684$ ,  $P = .0008$ ) and negatively with cholesterol ( $r = -0.782$ ,  $P = .0009$ ) and PC activity ( $r = -0.588$ ,  $P = .009$ ). Elevation in serum bilirubin, but not alanine aminotransferase, was negatively correlated with MA, G, and angle ( $r = -0.598$ ,  $P = .008$ ;  $r = -0.532$ ,  $P = .019$ ;  $r = -0.637$ ,  $P = .002$ , respectively). There was no correlation between MA and PCV or platelet count ( $r = 0.287$ ,  $P = .221$ ;  $r = 0.178$ ,  $P = .262$ , respectively), but MA correlated with fibrinogen (0.644,  $P = .044$ ) and PC activity (0.752, 0.012).

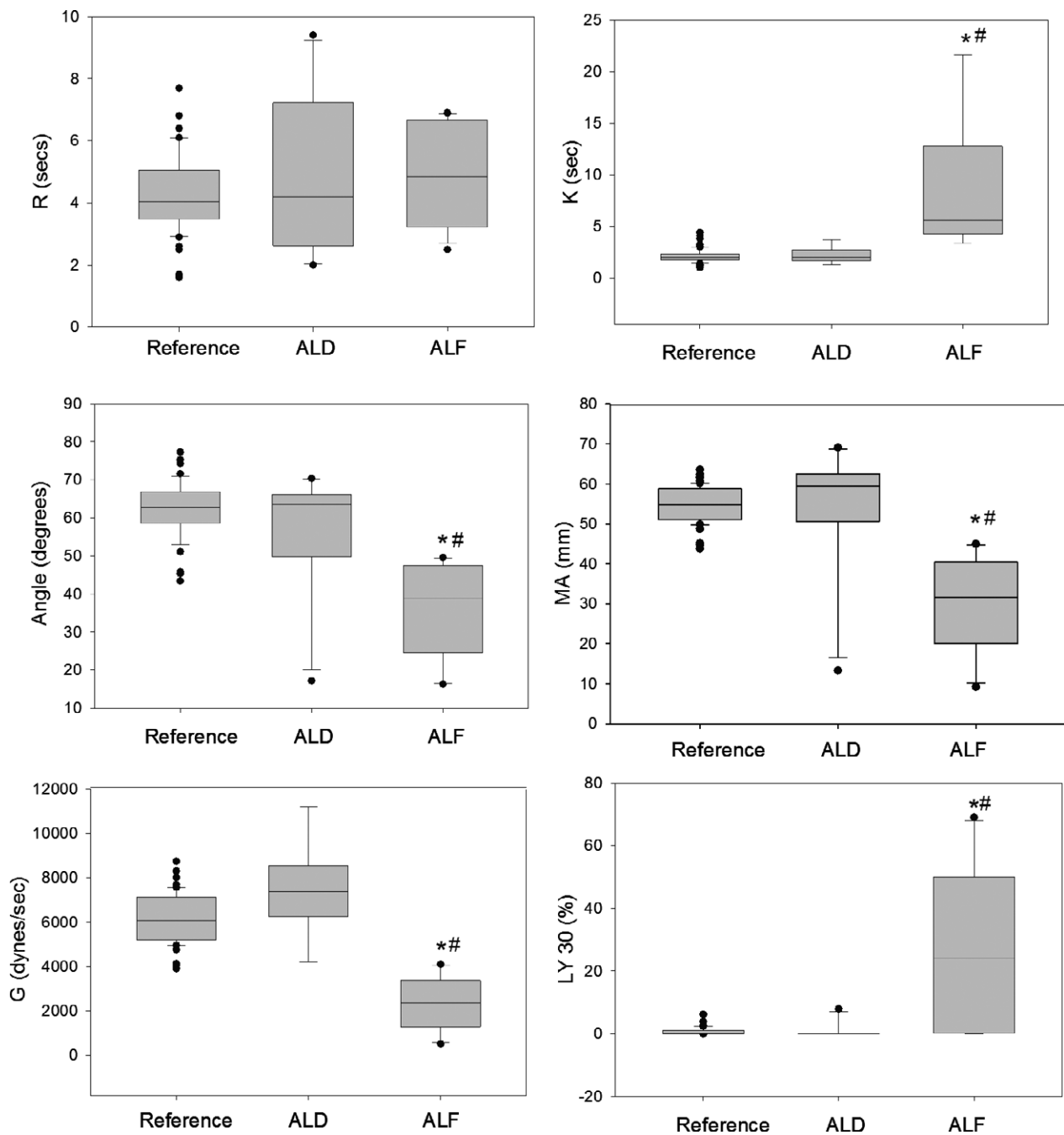
## Discussion

Our analysis showed that dogs with ALD have derangements in TEG variables as well as pro- and anticoagulants. Our data suggest that early in disease when hepatic synthetic capacity is not severely compromised (minimal prolongations in PT and aPTT), dogs with ALD are normocoagulable on TEG. As synthetic capacity decreases (moderate to severe PT and aPTT prolongations, decreases in PC activity and fibrinogen), dogs develop ALF and become hypocoagulable on TEG with increases in K and decreases in MA, angle, and G. In addition, some dogs with ALF become hyperfibrinolytic.

We found that most dogs with ALD were classified as either hypocoagulable (11/21) or normocoagulable (8/21) on TEG analysis. When comparing TEG coag-

ulation status with conventional coagulation testing (PT, aPTT, and platelet count) typically used to assess bleeding risk, we discovered some important inconsistencies. Four dogs with prolongation in PT, aPTT, or both (2/4 had platelet counts below 50,000/ $\mu L$ ) that would have been interpreted as having bleeding tendencies, actually were normocoagulable on TEG analysis. These dogs had normal PCVs and thus decreases in blood viscosity likely did not contribute to higher than normal MA values.<sup>25</sup> Conversely, 1 dog with normal PT and platelet count that would have been interpreted as not having a bleeding tendency, was hypocoagulable on TEG. None of the dogs with low AT or PC activity were hypercoagulable as might be predicted from loss of these anticoagulants. In fact, 4/9 and 4/6 dogs with low AT and PC activity, respectively, were labeled as hypocoagulable by TEG. Overall, we found that TEG evaluation was discordant with conventional coagulation testing 25% of the time. It remains to be determined if TEG might be a more accurate way to assess the complex balance of hemostasis that occurs in dogs with ALD and thus be useful to predict bleeding or thrombotic tendencies.

Thromboelastography has long been used in human hepatology as a bedside test to evaluate coagulation status in liver transplant patients and guide therapeutic intervention.<sup>5–7</sup> A recent study indicated that TEG accurately predicted bleeding tendencies in cirrhotic patients.<sup>9</sup> Although no studies have been done in dogs with liver disease, TEG has predicted bleeding tendencies in dogs with nonhepatic disorders. In 1 study, the G value calculated from tissue factor-activated TEG had a positive predictive value of 89% and a negative predictive value of 98% in identifying bleeding tendencies.<sup>27</sup> In a separate study limited to greyhounds, MA, angle, and G predicted postoperative bleeding tendencies.<sup>28</sup> Lastly, in a recent study, TEG was reliable in identifying thrombocytopenic dogs to be at a low risk of bleeding.<sup>29</sup> Prospective studies that evaluate TEG in a large number of dogs and carefully document bleeding and thrombotic tendencies are needed before the value of TEG in accessing coagulation status in dogs with ALD is affirmed.

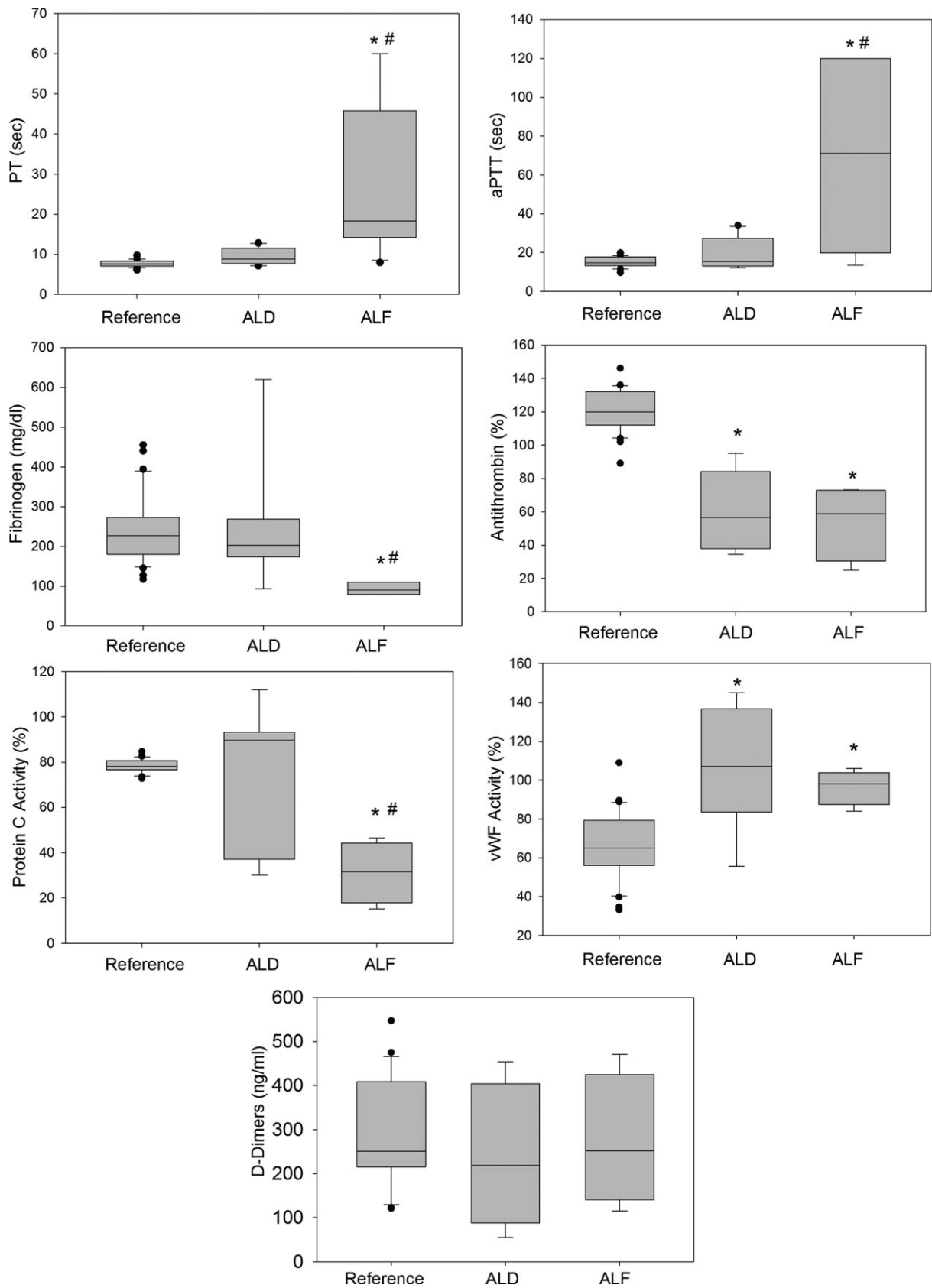


**Fig 1.** Comparison of TEG results in normal dogs (reference) and in dogs with acute liver disease (ALD) or acute liver failure (ALF). \* means significantly different than reference value; # indicates significantly different from value in dogs with ALD.

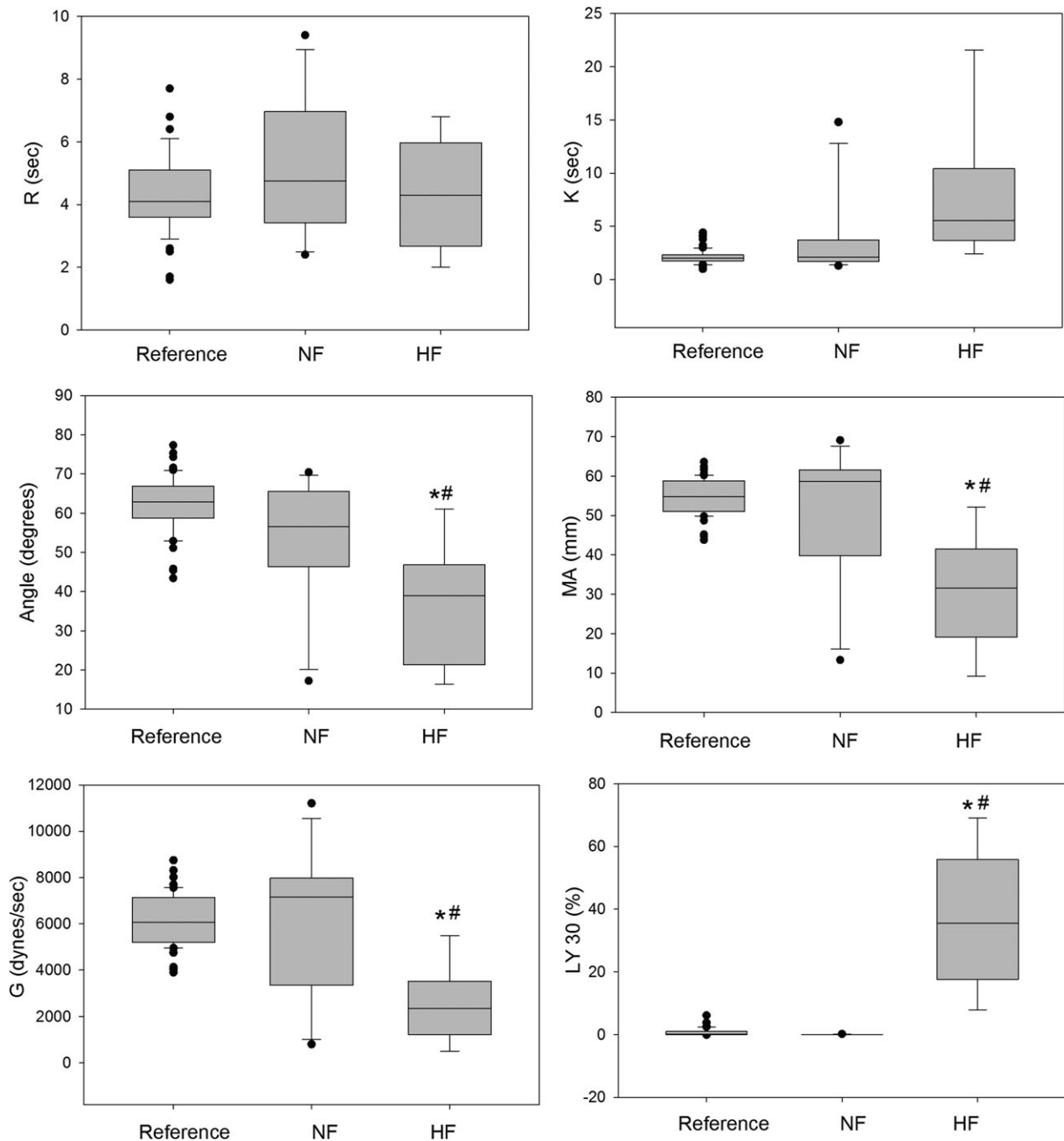
Although TEG analysis has been used to demonstrate hyperfibrinolysis in dogs with SIRS and disseminated intravascular coagulation (DIC),<sup>26,30</sup> ours is the first report of an association of hyperfibrinolysis with ALD in dogs. Hyperfibrinolysis can be primary or secondary.<sup>31</sup> Primary hyperfibrinolysis occurs independently of the action of coagulation and is associated with conditions that cause tissue plasminogen activator (tPA) to be released from the endothelium (eg, trauma, neoplasia, treatment with thrombolytic drugs and secondary to the presence of ascites). Secondary hyperfibrinolysis

is associated with activation of the coagulation system as seen in SIRS, sepsis, and DIC.<sup>26,30</sup> Because of the liver's essential role in the production of both pro- and anticoagulants and regulators of fibrinolysis, it can be challenging to determine if primary or secondary fibrinolysis is occurring in patients with ALD.<sup>31</sup>

Several factors suggest that the dogs in this study did not have secondary hyperfibrinolysis. None of the hyperfibrinolytic dogs had increased d-dimers, most were not thrombocytopenic and none had changes in RBC morphology (eg, schistocytes) or evidence of end-organ



**Fig 2.** Comparison of conventional plasma-based coagulation testing in normal dogs (reference) and in dogs with acute liver disease (ALD) or acute liver failure (ALF). \* means significantly different than reference value; # indicates significantly different from value in dogs with ALD

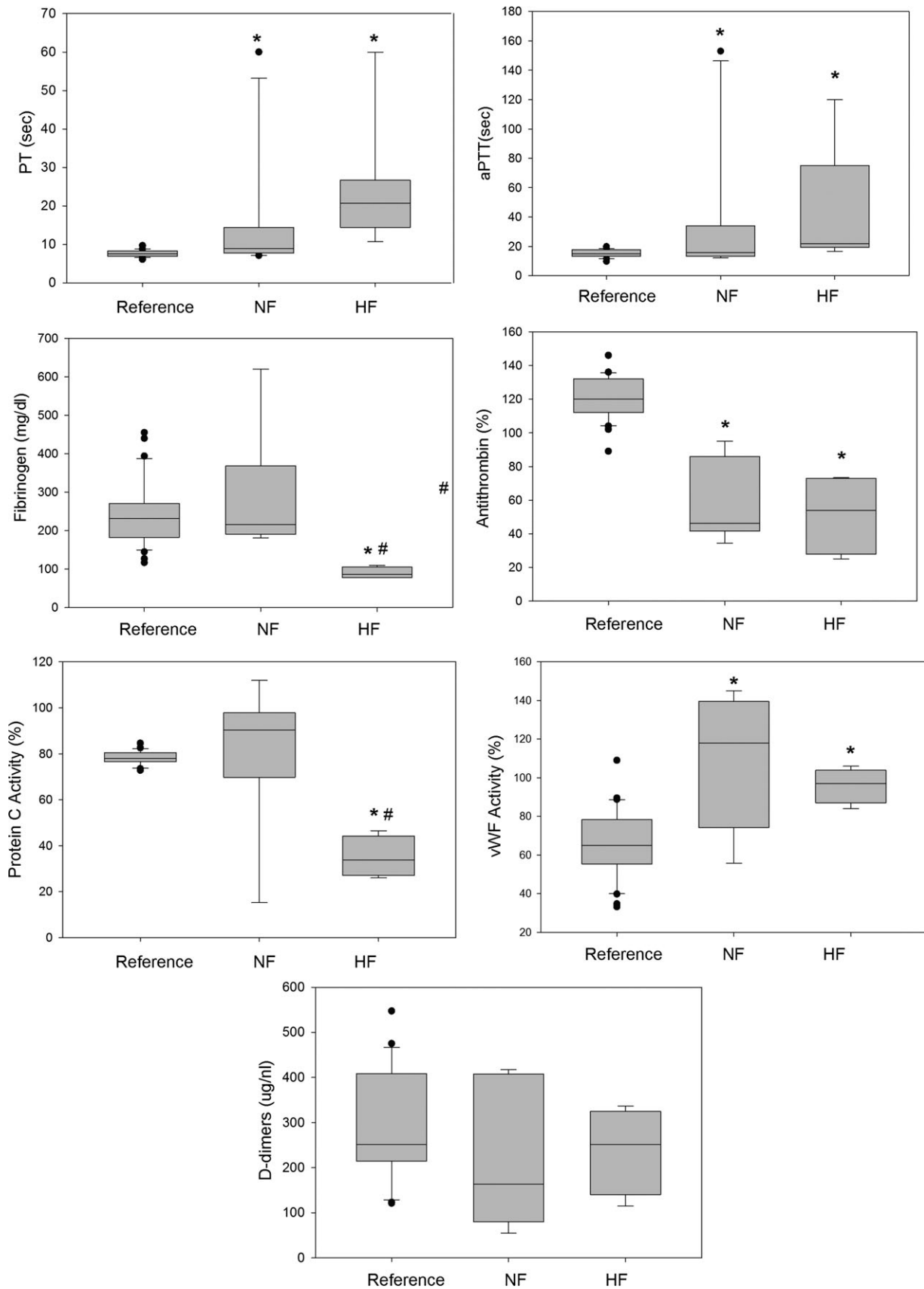


**Fig 3.** Comparison of TEG variables in normal dogs (reference) and in dogs with (HF) and without (NF) hyperfibrinolysis. \* means significantly different than reference value; # indicates significantly different from value in NF dogs

damage (eg, thrombi), changes that typically are used to diagnose DIC. Although 4/8 (50%) of the hyperfibrinolytic dogs had evidence of SIRS, 4/13 (30%) of the dogs without hyperfibrinolysis also had criteria consistent with SIRS.<sup>30</sup> Active infection was not documented in any of the dogs in the study, although only a few bacterial cultures were done (3/21) and most dogs were treated empirically with broad-spectrum antibiotics. Therefore, the role of sepsis in the coagulation changes remains unclear. White blood cell count positively

correlated with LY30 in dogs with ALD, suggesting that inflammation played a role in the hyperfibrinolytic state.

Several factors could have contributed to what is likely a primary hyperfibrinolytic state in dogs with ALD. One contributing factor could have been the absorption of fibrinolytic factors from ascitic fluid.<sup>31,32</sup> Two of 8 (25%) of the hyperfibrinolytic dogs had ascites, but 5 additional dogs with ascites were not hyperfibrinolytic so this is unlikely to be the sole cause.



**Fig 4.** Comparison of conventional coagulation testing in normal dogs (reference) and in dogs with (HF) and without (NF) hyperfibrinolysis. \* means significantly different than reference value; # indicates significantly different from value in NF dogs.



Excesses of tPA or urokinase-like plasminogen activator (uPA), which are cleared by the liver, or decreases in plasminogen activator inhibitor-1, thrombin activatable fibrinolysis inhibitor, or antiplasmin, which are produced by the liver, also could cause a primary hyperfibrinolytic state. Dogs are known to have increased fibrinolytic capacity related to higher uPA activity,<sup>33</sup> which could be a contributory factor predisposing dogs with ALD to hyperfibrinolysis. Endothelial- or leukocyte-derived microparticles released during ALD may generate a range of plasmin activity and contribute to excess fibrinolysis in humans.<sup>34,35</sup> The observation that 1 bleeding hyperfibrinolytic dog in this study responded to infusion of protease inhibitor (aminocaproic acid) suggests that a primary state of hyperfibrinolysis characterized by excess plasmin activity was occurring. The ability of TEG analysis to detect hyperfibrinolysis is clinically relevant because excessive activation of the fibrinolytic pathway could result in delayed bleeding from mucosal surfaces. In human trauma patients, primary hyperfibrinolysis as determined by a LY30 value >3% predicts the need for blood transfusion.<sup>36</sup>

In our population of dogs, hyperfibrinolysis and hypocoagulability were more common in dogs with ALF. This was reflected in the observation that LY30 was positively correlated with prolongations in PT and aPTT, increases in serum bilirubin concentration, and decreases in cholesterol concentration, all of which serve as biomarkers of disease severity in ALD.<sup>1-4,12</sup> One unique finding in the dogs with hyperfibrinolysis was low PC activity which was not seen in nonhyperfibrinolytic dogs. Serum bilirubin concentration, but not serum transaminase activity, was significantly negatively correlated with MA, G, and angle. This negative correlation fits with the observation that TEG parameters associated with hypocoagulability occur as hepatic synthetic failure ensues. In humans, changes in MA also have been correlated with serum bilirubin concentration and the severity of hepatic injury on biopsy.<sup>6</sup>

Two dogs with ALD had TEG parameters suggestive of a hypercoagulable state, which is common in people with ALD.<sup>6</sup> Dogs with ALD had several biochemical alterations that could predispose them to hypercoagulability, such as decreases in AT and PC activity and increases in vWF activity. Unfortunately, a full coagulation panel was not done in these 2 hypercoagulable dogs. Hypercoagulability in humans with ALD is associated with increases in fibrinogen concentration and evidence of endothelial dysfunction (ie, increases in Factor VIII and vWF activity) as well as decreases in ADAMTS 13 (zinc-containing metalloprotease enzyme that cleaves vWF).<sup>6</sup> Some dogs in this study and in a previous study of acute hepatitis<sup>37</sup> had increases in Factor VIII or vWF activity that could contribute to a hypercoagulable state.

Our study had several limitations. Our population of dogs was not homogenous with respect to underlying cause. Because of fear of bleeding tendencies, only a few of the dogs had liver biopsies performed to determine etiology. Additionally, we used a kaolin-activated TEG assay, and results of a tissue factor-activated TEG

assay may differ. As such, future studies should be designed to compare the results of these 2 assays. This study was a pilot study with a small sample size of 21 dogs with incomplete datasets; thus, future studies should include a larger population with complete datasets to determine if our results are repeatable. Future prospective studies should include a more comprehensive panel of coagulation and fibrinolytic factors (eg, Factor VIII, tPA, uPA, plasminogen activator inhibitor-1, thrombin activatable fibrinolysis inhibitor, alpha-plasmin, thrombin generation tests, thrombin-anti-thrombin complexes) as well as characterization of microparticle biology to determine the pathophysiology of an ALD-induced hyperfibrinolytic state in dogs. In humans, endogenous heparinoids are generated in ALF as a consequence of endothelial damage and can contribute to bleeding tendencies, and TEG analysis is capable of evaluating this situation.<sup>38</sup> Future studies with TEG analysis should be designed to include the addition of heparinase I, which cleaves heparin-like compounds, to reveal the presence of a heparin-like effect. Although the majority of our patients with hyperfibrinolysis had significant prolongations of LY30 (median, 29.3%; range, 7.9–69%), one of the limitations of TEG analysis is its inability to differentiate, at low levels of LY30, between genuine fibrinolysis and platelet-mediated effects.

In conclusion, TEG analysis in this study suggested that conventional plasma-based coagulation testing may overestimate decreased functionality of the coagulation system in dogs with early ALD, but as severe functional hepatic impairment ensues, both conventional coagulation testing and TEG indicate the onset of a hypocoagulable state. TEG analysis identified the presence of a hyperfibrinolytic state that cannot be detected by conventional plasma-based testing. This observation is clinically relevant because hyperfibrinolysis can be associated with bleeding tendencies and can be readily corrected with antiprotease treatment and repletion of fibrinogen. Future studies should be aimed at delineating the role of TEG analysis in the identification and management of the hemostatic abnormalities accompanying ALD in dogs.

---

## Footnotes

<sup>a</sup> ACL Elite Analyzer, Beckman Coulter, Brea, CA

<sup>b</sup> TEG 500 Thromboelastograph, Haemonetics Corp, Braintree, MA

---

## Acknowledgments

This study was supported by a grant from the Companion Animal Health Fund at the Cummings School of Veterinary Medicine at Tufts University.

*Conflict of Interest Declaration:* Authors disclose no conflict of interest.

*Off-label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

## References

- Dereszynski DM, Center SA, Randolph JF, et al. Clinical and clinicopathologic features of dogs that consumed foodborne hepatotoxic aflatoxins: 72 cases (2005–2006). *J Am Vet Med Assoc* 2008;232:1329–1337.
- Dunayer EK, Gwaltney-Brant SM. Acute hepatic failure and coagulopathy associated with xylitol ingestion in eight dogs. *J Am Vet Med Assoc* 2006;229:1113–1117.
- Toulza O, Center SA, Brooks MB, et al. Evaluation of plasma protein C activity for detection of hepatobiliary disease and portosystemic shunting in dogs. *J Am Vet Med Assoc* 2006;229:1761–1771.
- Prins M, Schellens CJ, van Leeuwen MW, et al. Coagulation disorders in dogs with hepatic disease. *Vet J* 2010;185:163–168.
- Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease: Evidence and clinical consequences. *Blood* 2010;116:878–885.
- Stravitz RT. Potential applications of thromboelastography in patients with acute and chronic liver disease. *Gastroenterol Hepatol* 2012;8:513–520.
- Northup PG, Caldwell SH. Coagulation in liver disease: A guide for the clinician. *Clin Gastroenterol Hepatol* 2013;11:1064–1074.
- Lee WM, Stravitz RT, Larson AM. Introduction to the revised AASLD position paper on acute liver failure 2011. *Hepatology* 2012;55:965–967.
- Chau TN, Chan YW, Patch D, et al. Thromboelastographic changes and early rebleeding in cirrhotic patients with variceal bleeding. *Gut* 1998;43:267–271.
- Papatheodoridis GV, Patch D, Webster GJ, et al. Infection and hemostasis in decompensated cirrhosis: A prospective study using thromboelastography. *Hepatology* 1999;29:1085–1090.
- Stravitz RT, Lisman T, Luketic VA, et al. Minimal effects of acute liver injury/acute liver failure on hemostasis as assessed by thromboelastography. *J Hepatol* 2012;56:129–136.
- Kavanagh C, Shaw S, Webster CR. Coagulation in hepatobiliary disease. *J Vet Emerg Crit Care (San Antonio)* 2011;21:589–604.
- Mayhew PD, Savigny MR, Otto CM, et al. Evaluation of coagulation in dogs with partial or complete extrahepatic biliary tract obstruction by means of thromboelastography. *J Am Vet Med Assoc* 2013;242:778–785.
- Kelley D, Lester C, DeLaforcade A, et al. Thromboelastographic evaluation of dogs with congenital portosystemic shunts. *J Vet Intern Med* 2013;27:1262–1267.
- Rose L, Dunn ME, Bédard C. Effect of canine hyperadrenocorticism on coagulation parameters. *J Vet Intern Med* 2013;27:207–211.
- Goodwin LV, Goggs R, Chan DL, et al. Hypercoagulability in dogs with protein losing enteropathy. *J Vet Intern Med* 2011;25:273–277.
- Donahue SM, Brooks M, Otto CM. Examination of hemostatic parameters to detect hypercoagulability in dogs with severe protein-losing nephropathy. *J Vet Emerg Crit Care (San Antonio)* 2011;21:346–355.
- Fenty RK, DeLaforcade AM, Shaw SE, et al. Identification of hypercoagulability in dogs with primary immune-mediated hemolytic anemia by means of thromboelastography. *J Am Vet Med Assoc* 2011;238:463–467.
- O'Marra SK, Shaw SP, DeLaforcade AM. Investigating hypercoagulability during treatment for immune-mediated thrombocytopenia: A pilot study. *J Vet Emerg Crit Care (San Antonio)* 2012;22:126–130.
- Otto CM, Rieser TM, Brooks MB, et al. Evidence of hypercoagulability in dogs with parvoviral enteritis. *J Am Vet Med Assoc* 2000;217:1500–1504.
- Kristensen AT, Wiinberg B, Jessen LR, et al. Evaluation of human recombinant tissue factor-activated thromboelastography in 49 dogs with neoplasia. *J Vet Intern Med* 2008;22:140–147.
- Vilar P, Couto CG, Westendorf N, et al. Thromboelastographic tracings in retired racing greyhounds and in non-greyhound dogs. *J Vet Intern Med* 2008;22:374–379.
- Polson J, Lee WM. AASLD position paper: The management of acute liver failure. *Hepatology* 2005;41:1179–1197.
- Hanel RM, Chan DL, Conner B, et al. Systematic evaluation of evidence on veterinary viscoelastic testing part 4: Definitions and data reporting. *J Vet Emerg Crit Care (San Antonio)* 2014;24:47–56.
- Smith SA, McMichael MA, Gilor S, et al. Correlation of hematocrit, platelet concentration, and plasma coagulation factors with results of thromboelastometry in canine whole blood samples. *Am J Vet Res* 2012;73:789–798.
- Bauer N, Moritz A. Coagulation response in dogs with and without systemic inflammatory response syndrome-preliminary results. *Res Vet Sci* 2013;94:122–131.
- Wiinberg B, Jensen AL, Rozanski E, et al. Tissue activatable thromboelastography correlates to clinical signs of bleeding in dogs. *Vet J* 2009;179:121–129.
- Vilar-Saavedra P, Stingle N, Iazbik C, et al. Thromboelastographic changes after gonadectomy in retired racing greyhounds. *Vet Rec* 2011;169:99.
- Bucknoff MC, Hanel RM, Marks SL, et al. Evaluation of thromboelastography for prediction of clinical bleeding in thrombocytopenic dogs after total body irradiation and hematopoietic cell transplantation. *Am J Vet Res* 2014;75:425–432.
- Vilar-Saavedra P, Hosoya K. Thromboelastographic profile for a dog with hypocoagulable and hyperfibrinolytic phase of DIC. *J Small Anim Prac* 2011;52:656–659.
- Ferro D, Celestini A, Violi F. Hyperfibrinolysis in liver disease. *Clin Liver Dis* 2009;13:21–31.
- Zoia A, Augusto M, Drigo M, et al. Evaluation of hemostatic and fibrinolytic markers in dogs with ascites attributable to right-sided congestive heart failure. *J Am Vet Med Assoc* 2012;241:1336–1343.
- Lang IM, March JJ, Konopka RG, et al. Factors contributing to increased vascular fibrinolytic activity in mongrel dogs. *Circulation* 1993;87:1990–2000.
- Stravitz RT, Bowling R, Bradford RL, et al. Role of procoagulant microparticles in mediating complications and outcome of acute liver injury/acute liver failure. *Hepatology* 2013;58:304–313.
- Herring JM, McMichael MA, Smith SA. Microparticles in health and disease. *J Vet Intern Med* 2013;27:1020–1033.
- Chapman MP, Moore EE, Ramos CR, et al. Fibrinolysis greater than 3% is the critical value for initiation of antifibrinolytic therapy. *J Trauma Acute Care Surg* 2013;75:961–967.
- Badylack SF, Dodds WJ, Van Vleet JF. Plasma coagulation factor abnormalities in dogs with naturally occurring hepatic disease. *Am J Vet Res* 1983;44:2336–2340.
- Senzolo M, Agarwal S, Zappoli P, et al. Heparin-like effect contributes to the coagulopathy in patients with acute liver failure undergoing liver transplantation. *Liver Int* 2009;29:754–759.