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Changes in the coagulation parameters in dogs with protein-losing enteropathy between before and after treatment

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ABSTRACT. Protein-losing enteropathy (PLE) is known to induce hypercoagulability and resultant thromboembolism in dogs. We hypothesized that hypercoagulability would improve if remission was obtained in dogs with PLE after treatment. This study aimed to evaluate the changes in the coagulation parameters after treatment in dogs diagnosed with PLE. As coagulation parameters, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, thrombin-antithrombin complex (TAT), D-dimer, and antithrombin (AT) were measured. In addition to these parameters, rotational thromboelastometry (ROTEM), which evaluates the comprehensive coagulation and fibrinolysis reactions of whole blood, was conducted and the data of clotting time (CT), clot formation time (CFT), α angle (α), maximum clot firmness (MCF) and lysis index at 60 min (LI60) were obtained. Eleven of the 14 dogs diagnosed with PLE were classified as responders to the treatment based on the changes in their plasma albumin (ALB) concentration after treatment. Significant increase in CFT and decrease of α and MCF indicating the resolution of hypercoagulability were found after treatment in responder dogs; however, there was no significant change in the coagulation and fibrinolysis parameters other than those measured by ROTEM. This study demonstrated that the hypercoagulability detected by ROTEM was significantly improved after treatment in dogs with PLE.

KEY WORDS: coagulation, dog, protein-losing enteropathy, treatment

Protein-losing enteropathy (PLE) is a syndrome characterized by hypoalbuminemia caused by excessive plasma protein loss from the intestine. In dogs, PLE is associated with chronic small intestinal disorders including chronic enteritis, intestinal lymphangiectasia, and small- and large-cell gastrointestinal lymphomas [29, 33]. Treatments for PLE include glucocorticoids, immunosuppressive drugs (e.g., azathioprine, cyclosporine, and chlorambucil), and dietary management (low-fat or ultra-low-fat diet) [7, 8, 12, 29, 31, 38, 39]. The median survival time of dogs with PLE is variable [2, 3, 7, 11, 29, 31, 39]. The prognosis of PLE depends on the underlying disease: the overall survival longest in chronic enteritis or intestinal lymphangiectasia, followed by small-cell lymphoma and shortest in large-cell lymphoma [29].

A previous study reported that dogs with PLE were shown to be in a hypercoagulable state in the thromboelastography (TEG) analysis when compared with healthy controls [14]. Although the mechanism has not been clarified yet, both of the decrease in plasma antithrombin (AT) activity [14] and the underlying diseases such as chronic enteritis and intestinal lymphoma [21, 25] are conceivably related to hypercoagulability in dogs with PLE. Furthermore, thromboembolism is known as one of the complications of PLE in dogs [10, 13, 17, 42]. In a case series of thromboembolism in dogs with PLE, 8 dogs were shown to have thrombi in pulmonary arteries/vessels and the thromboembolism was diagnosed in 7.1% of the dogs with PLE [17]. Therefore, pulmonary thromboembolism is considered to be one of the serious complications in dogs with PLE.

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Glucocorticoid, a commonly used drug for the treatment of PLE, is known to cause hypercoagulability in dogs [36, 37]. The administration of glucocorticoids in dogs with PLE may exacerbate hypercoagulability. A previous report mentioned that there was no significant difference in the coagulation parameters between before and after treatment for PLE, however, the duration of treatment was short and the changes in coagulation parameters was not evaluated in detail [14]. We assume that it takes several weeks to change the coagulation parameters after treatment of PLE and the change would be different between treatment responders and non-responders. It is necessary to evaluate the changes in coagulation parameters after several weeks of treatment and classify the dogs based on the response to treatment of PLE.

In addition to the conventional coagulation profiles, we employed a rotational thromboelastometry (ROTEM; ROTEM Delta, Munich, Germany) in the present study. ROTEM is a modern modification of TEG technology, which measures the viscoelastic properties of whole blood during clot formation, stabilization, and fibrinolysis [1, 22, 41]. ROTEM provides automated pipetting while TEG needs manual pipetting. Moreover, ROTEM is less susceptible to vibration in contrast to the TEG which is very sensitive to vibration [1, 22, 41]. Based on these differences, ROTEM is presumed to be less likely to cause artifacts compared to TEG.

We hypothesized that when the plasma albumin (ALB) level increased after the treatments for PLE, the plasma AT activity would increase and subsequently the hypercoagulable state would also ameliorate. This study aimed to evaluate the changes in coagulation and fibrinolysis parameters including those measured by ROTEM between before and after treatment in dogs with PLE.

MATERIALS AND METHODS

Dogs with PLE

Dogs diagnosed with PLE from October 2018 to October 2019 at the Veterinary Medical Center of The University of Tokyo and whose coagulation and fibrinolysis parameters before and after treatment for PLE were evaluable were included in this study. PLE was diagnosed when all of the following conditions were observed: (1) history of chronic gastrointestinal symptoms, (2) hypoalbuminemia (<2.6 g/dl), (3) histopathological diagnosis of gastrointestinal disease known to be associated with PLE, and (4) absence of other causes of hypoalbuminemia. Absence of proteinuria was confirmed by a negative urine dipstick or urine protein creatinine ratio <0.5. Cases were excluded if some anticoagulant or antiplatelet were used before and during the treatments for PLE and its underlying disease. Written consent was obtained from the owners of all dogs included in the study.

For histological examination, mucosal biopsy specimens were obtained by gastrointestinal endoscopy, and the dogs were classified into three groups (chronic enteritis, small-cell lymphoma, and large-cell lymphoma), as previously described [29]. Biopsy samples were fixed in 10% neutral buffered formalin and processed for routine histopathologic analysis after paraffin embedding. Hematoxylin and eosin-stained sections were evaluated for diagnosis. Chronic enteritis was diagnosed according to the histopathological standards of the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group [9]. We identified intestinal lymphangiectasia from the findings of marked lacteal dilation of the villous lamina propria [9]. Small-cell and large-cell lymphomas were diagnosed according to the World Health Organization lymphoma diagnostic criteria in conjunction with the descriptions in recent studies on small-cell lymphoma in dogs [5, 6, 27, 29].

Information including breed, sex, age, body weight, and body condition scores (BCS; scale 1–5) was obtained in each dog. Severity of the clinical signs was assessed using the canine inflammatory bowel disease activity index (CIBDAI) [18] and the canine chronic enteropathy clinical activity index (CCECAI) [3]. The CIBDAI was calculated with scores for six clinical signs, including attitude/activity, appetite, vomiting, stool consistency, stool frequency, and weight loss. The CCECAI scoring index included hypoalbuminemia, assessment of ascites, peripheral edema and pruritis in addition to the CIBDAI scores.

For the evaluation of response to treatment, the dogs were divided into two groups: responders and non-responders based on their plasma ALB concentration, CIBDAI and CCECAI. The level of hypoalbuminemia was categorized into 5 levels (Score 1: ≥2.6 g/dl, Score 2: 2.0–2.5 g/dl, Score 3: 1.5–1.9 g/dl, Score 4: 1.2–1.4 g/dl, Score 5: ≤1.1 g/dl) based on a previous report [3] with minor modifications. The score of normoalbuminemia (≥2.6 g/dl) was added to the score of a previous report to assess the improvement of mild hypoalbuminemia (2.0–2.5 g/dl) to normoalbuminemia (≥2.6 g/dl). The CIBDAI and CCECAI were categorized into four (Score 1: 0–3, Score 2: 4–5, Score 3: 6–8, Score 4: ≥9) and five (Score 1: 0–3, Score 2: 4–5, Score 3: 6–8, Score 4: 9–11, Score 5: ≥12) levels as previously reported [3, 18]. If score decrease ≥1 was observed after treatment, the dog was classified as a responder, and if the score was unchanged or increased, the dog was classified as a non-responder.

Coagulation parameters

Blood samples were collected from the jugular vein using a 23-gauge needle, and collected blood was dispensed into 3.2% sodium citrate for the coagulation tests (blood-to-citrate ratio: 9:1). Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen concentration were measured using a dry-hematology analyzer (COAG2NV, ERMA Inc., Tokyo, Japan). Thrombin-antithrombin complex (TAT) level was determined by chemiluminescent enzyme immunoassay using Pathfast (LSI Medience Corp., Tokyo, Japan), which was validated in dogs [19]. D-dimer concentration and plasma AT activity were determined in a commercial laboratory (Sanritsu Zelkova Laboratory, Kawasaki, Japan) by latex particle-enhanced immunoturbidimetric assay and synthetic chromogenic substrate method, respectively.

ROTEM analysis was performed according to the manufacturer's instructions, and the analyses were performed for 90 min. Two different assays were tested for each sample, ex-TEM and in-TEM. For the ex-TEM assay, the blood was recalcified with a

concentrated calcium chloride reagent (star-TEM reagent) and activated by the tissue factor reagent (ex-TEM reagent). In contrast, the in-TEM profile was activated by the ellagic acid reagent (in-TEM reagent) after recalcification. The following parameters were assessed for each profile: clotting time ([CT], measured in sec), which is the lag period from the initiation of the reaction until an amplitude reaches 2 mm, clot formation time ([CFT], measured in sec), which is the duration necessary for the amplitude to increase from 2 to 20 mm, α angle ([α], measured in degrees), which is the angle of change of the amplitude throughout the CFT, and maximum clot firmness ([MCF], measured in mm), which is the greatest amplitude observed during the measurement (Fig. 1). In addition, the lysis index at 60 min ([LI60], measured in %), which is the percentage of remaining clot stability in relation to the MCF following the 60 min after CT was evaluated as indicator for fibrinolysis. These ROTEM parameters, CT, CFT, α, MCF and LI60, correspond to r, K, α, maximum amplitude and LY60 in TEG, respectively. Prior to the application to dogs with PLE, ROTEM analysis was carried out in 15 clinically healthy dogs judged from physical examination, complete blood count and plasma biochemistry (10 castrated males, 3 spayed females, and 2 intact females; median age 4.7 years old, range 1.0–14.7 years old; breed- 8 Beagles, 6 Labrador Retrievers, and 1 mixed breed). Although beagles are predisposed to congenital factor VII deficiency which can cause hypocoagulability and prolongation of PT [4], all beagles included in the present study had normal PT value and congenital factor VII deficiency was ruled out.

Statistical analysis

The statistical analysis was carried out using RStudio v.1.1.463 (RStudio, Boston, MA, USA). The CIBDAI, CCECAI, plasma ALB concentration, and hematological parameters at the time of diagnosis between dogs treated with or without glucocorticoids were compared using the Mann-Whitney U test. The values of ROTEM parameters in dogs with PLE before treatment were compared with those of controls using the Mann-Whitney U test. The CIBDAI, CCECAI, and hematological parameters between baseline and after treatment were compared using the Wilcoxon signed-rank test in responders and non-responders, respectively. A P value <0.05 was considered statistically significant.

RESULTS

Dogs with PLE

Fourteen dogs with PLE were included in this study. The breeds were as follows: 3 Yorkshire Terriers, 2 Miniature Dachshunds, 2 Shibas, and 1 each of Beagle, French Bulldog, Jack Russell Terrier, Miniature Pinscher, Pembroke Welsh Corgi, Toy Poodle and mixed breed dog. There were 6 castrated males, 7 spayed females, and 1 intact female. The median age at the time of diagnosis of PLE was 9.1 years old (range: 3.8–13.5 years old). The median body weight was 5.1 kg (range: 1.7–9.7 kg), and the mean BCS was 2.5 (range: 1–4). Clinical signs observed in the 14 dogs included weight loss (9 dogs), diarrhea (9), anorexia (6), lethargy (5), ascites (7), and vomiting (2). Based on the histopathological examination, 8 dogs were classified as chronic enteritis, 4 as small-cell lymphoma, and 2 as large-cell lymphoma. Intestinal lymphangiectasia was observed in 6 of the 8 dogs diagnosed with chronic enteritis. A computed tomography examination was conducted in 5 of the 14 dogs, and none of the dogs in the present study had thrombus.

Coagulation parameters in dogs with PLE before treatment

The values of PT, aPTT, fibrinogen and AT in the 14 dogs with PLE were outside the reference ranges in 1/14 (7%), 3/14 (21%), 5/14 (36%), and 14/14 (100%) dogs, respectively. TAT, and D-dimer concentrations were above the reference ranges in 6/14 (43%) and 8/14 (57%) dogs, respectively. The comparison of ROTEM parameters between control dogs and dogs with PLE before treatment revealed that dogs with PLE were significantly hypercoagulable in all ROTEM parameters (CT, CFT, α and MCF in both in-TEM and ex-TEM; all P<0.05), whereas no significant difference was observed in fibrinolysis indicator of LI60 (Table 1).

Glucocorticoid had been already administered in 7 dogs (chronic enteritis: 5 dogs and small-cell lymphoma: 2 dogs) before the
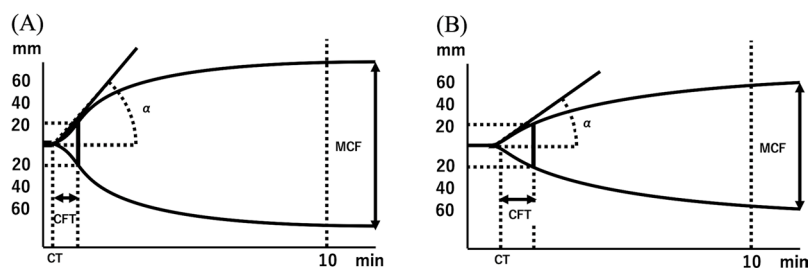
Fig. 1. Depiction of representative data of rotational thromboelastometry before (A) and after treatment (B) in a dog with protein-losing enteropathy. The x-axis represents time and the y-axis represents the amplitude of pin rotation which reflects clot firmness. The clotting time (CT) and clot formation time (CFT) are the times that takes for the tracing to reach the amplitudes of 2 and 20 mm, respectively. The α angle (α) is the angle of the slope representing acceleration of clot formation and maximum clot firmness (MCF) is the maximal amplitude of the tracing, representing the maximal strength of the clot. The increase in CT and CFT and decrease in α and MCF were observed after treatment. These results indicate the hypercoagulability was ameliorated in this dog after treatment.

enrollment to the study, with a median dosage of 1.0 mg/kg/day (range: 0.6–4.0 mg/kg/day). The median CCECAI, MCF (in-TEM) and MCF (ex-TEM) values at diagnosis in dogs that had been treated with glucocorticoid were 2 (range: 1–11), 73 mm (range: 67–83 mm) and 75 mm (range: 69–85 mm), respectively. The median CCECAI, MCF (in-TEM) and MCF (ex-TEM) values at diagnosis that had not been treated with glucocorticoids were 9 (range: 2–11), 66 mm (range: 62–76 mm) and 66 mm (range: 63–78 mm), respectively. These three parameters were significantly different between two groups (CCECEI, *P*=0.045; MCF (in-TEM), *P*=0.040; MCF (ex-TEM), *P*=0.046). No significant change was observed in the other parameters between the dogs that had been treated with or without glucocorticoids before the enrollment to the study (data not shown).

Changes in the coagulation parameters in dogs with PLE after treatment

The second set of blood collection was performed at a median of 45 days (range: 20–88 days) from the initiation of treatment. The eight dogs with chronic enteritis were treated with prednisolone (0.6–2.0 mg/kg, PO, q24 hr) (6 dogs), budesonide (0.27 mg/kg, PO, q24 hr) (1 dog), or chlorambucil (0.2 mg/kg, PO, q48 hr) (1 dog). All of the 4 dogs diagnosed with small-cell lymphoma were treated with prednisolone (0.6–1.0 mg/kg, PO, q24 hr). Two of the 4 dogs were also treated with chlorambucil (0.7 mg/kg, PO, q72 hr and 0.4 mg/kg, PO, q24 hr) and 1 of the 4 dogs were also treated with L-asparaginase (400 IU/kg, SC, once a week). The 2 dogs diagnosed with large-cell lymphoma were treated with prednisolone (0.6–1.7 mg/kg, PO, q24 hr) and L-asparaginase (400 IU/kg, SC, once a week). None of the dogs included in the present study received blood transfusion before and during treatment of PLE.

Eleven dogs were classified as treatment responders from the changes of ALB concentration. Of those, 7 dogs were classified as chronic enteritis, 2 as small-cell lymphoma, and 2 as large-cell lymphoma. The changes in coagulation parameters in the responders judged from the changes of ALB concentration are shown in Figs. 2 and 3. The pre-treatment median values of plasma ALB concentration, CIBDAI, and CCECAI were 1.7 g/dl (range: 1.1–2.5. g/dl), 4.0 (range: 1–11), and 8.0 (range: 1–11), respectively. After treatment, the median plasma ALB concentration was significantly increased to 2.8 g/dl (range: 1.8–3.6 g/dl, *P*=0.001), and the median CIBDAI and CCECAI were significantly decreased to 0 (range: 0–5, *P*=0.016), and 0 (range: 0–5, *P*=0.009), respectively. The baseline median CFT, α, and MCF in the ex-TEM profile were 82 sec (range: 31–112 sec), 74 degrees (range: 68–84 degrees), 70 mm (range; 63–85 mm), and median MCF in the in-TEM profile was 73 mm (range: 62–83 mm). After treatment, the median CFT significantly increased to 94 sec (range: 52–141 sec, *P*=0.007), and median α and MCF values significantly decreased to 71 degrees (range: 63–81 degrees, *P*=0.018) and 69 mm (range: 60–76 mm, *P*=0.045), respectively, in the ex-TEM profile. Figure 1 shows the ROTEM tracing in a dog with PLE before and after treatment. The low value of CT and CFT, and the high value of α and MCF indicate the hypercoagulability in ROTEM analysis [18, 35], and changes of these variables after treatment indicate the decrease of hypercoagulability. As for the in-TEM profile, the median MCF was significantly decreased to 66 mm (range: 51–77 mm, *P*=0.038). No significant changes were observed in PT, aPTT, fibrinogen, TAT, AT activity, D-dimer, and other ROTEM parameters (CT and LI60 in ex-TEM and in-TEM, CFT and α in in-TEM).

Three dogs were classified as non-responders from the ALB concentration after treatment. Of those, 1 dog was classified as chronic enteritis and 2 as small-cell lymphoma. Due to the small sample size, statistical analysis could not be conducted in this group. The value of CFT in the ex-TEM profile, which significantly increased in responders, was decreased in all of the non-responder dogs. However, no remarkable change was observed in α in ex-TEM, and MCF in in-TEM and ex-TEM. The changes in clinical, hematological, and coagulation parameters in responders and non-responders judged from change of the plasma ALB concentration are summarized in Tables 2 and 3.

Based on CIBDAI and CCECAI, 7 and 9 dogs were classified as responders, respectively. In the responders from CIBDAI and CCECAI, the baseline median CFT in the ex-TEM profile was 50 sec (range: 31–112 sec) and 69 sec (range: 31–112 sec), which significantly increased to 94 sec (range: 43–141 sec, *P*=0.031) and 94 sec (range: 43–141 sec, *P*=0.028) after treatment, respectively. No significant change was observed in other parameters including CFT in in-TEM, and α and MCF in ex-TEM, which significantly changed in responders judged from plasma ALB concentration (data not shown). Only 1 dog was classified as a non-responder from the CIBDAI and CCECAI. Six and 4 dogs were excluded from the analysis because CIBDAI and CCECAI at both the baseline and after treatment were within the reference range (0–3).

Table 1. The coagulation and fibrinolysis parameters measured by rotational thromboelastometry in control dogs and dogs with protein-losing enteropathy before treatment

Variables	Unit	Control (n=15)	PLE (n=14)	*P* value
CT (ex-TEM)	sec	61 (39–103)	43 (32–64)	<0.01
CFT (ex-TEM)	sec	126 (46–213)	76 (31–112)	<0.01
α (ex-TEM)	degree	65 (57–82)	75 (68–84)	<0.01
MCF (ex-TEM)	mm	59 (48–75)	70 (63–85)	<0.01
LI60 (ex-TEM)	%	98 (65–100)	97.5 (77–100)	0.859
CT (in-TEM)	sec	242 (198–381)	212 (166–281)	0.020
CFT (in-TEM)	sec	157 (65–239)	75 (38–135)	<0.01
α (in-TEM)	degree	60 (52–77)	75 (63–82)	<0.01
MCF (in-TEM)	mm	57 (49–71)	71 (62–83)	<0.01
LI60 (in-TEM)	%	99 (94–100)	99 (95–100)	0.928

Data are presented as median and range in the parentheses. PLE, protein-losing enteropathy; CT, clotting time; CFT, clot formation time; α, α angle; MCF, maximum clot firmness; LI60, lysis index 60.

DISCUSSION

The results of this study suggest that the hypercoagulable state resolves after treatment in parallel with the increase of the plasma ALB concentration in dogs with PLE. The CFT measured in ex-TEM significantly increased after treatment in responders based on plasma ALB concentration. The CFT refers to the duration until clot formation, and the low value of CFT indicates hypercoagulability [20, 41]. Therefore, the increase in CFT indicates the improvement of hypercoagulable state. The α measured in the ex-TEM and MCF in both in-TEM and ex-TEM significantly decreased in the responders. The values of α and MCF indicate the velocity of clot formation and the value of clot firmness, respectively, and the increase of α and MCF indicates hypercoagulability [20, 41]. Therefore, the decrease in α and MCF indicates the improvement of the hypercoagulable state. In contrast, in responders based on CIBDAI and CCECAI, only the MCF in the ex-TEM profile significantly decreased. Therefore, the improvement of clinical signs did not necessarily correspond with an improvement in the hypercoagulable state.

The significant reduction of hypercoagulability indicated from the parameters measured by ROTEM was observed; however, no significant change was observed in conventional coagulation tests including PT, aPTT, fibrinogen, TAT, AT and D-dimer. The decrease in PT, aPTT and fibrinogen are caused by excess consumption of coagulation factors [16, 32] and abnormality in these parameters was not found in the dogs with PLE enrolled in this study. Increase of D-dimer which indicates the amplitude of fibrinolysis is caused by excess fibrinolysis with the degradation of cross-linked fibrin by plasmin [23, 30]. Since the D-dimer
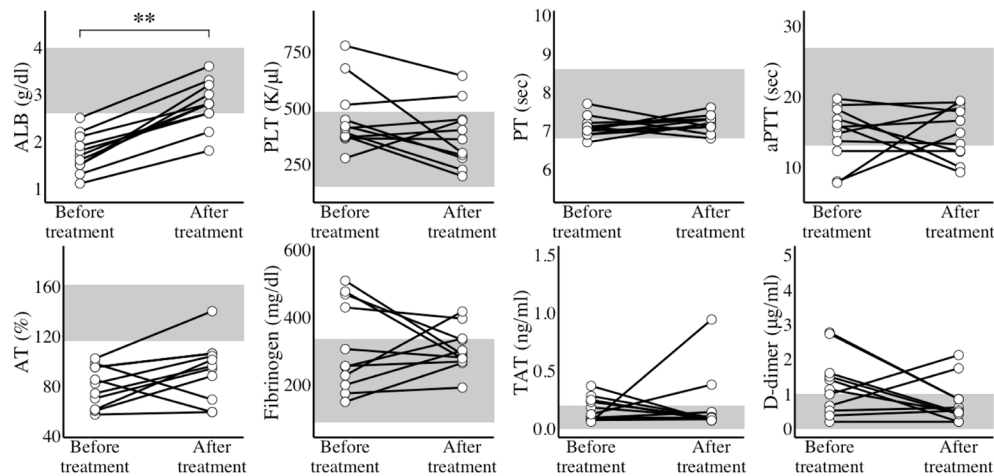
Fig. 2. Changes in the plasma albumin concentration and conventional coagulation parameters in dogs with protein-losing enteropathy (responders) after treatment. The gray background indicates the reference range of each coagulation parameter. Asterisks indicate significant difference between the values before and after treatment. ALB, albumin; PLT. platelet count; PT, prothrombin time; aPTT, activated partial thromboplastin time; AT, antithrombin; TAT, thrombin-antithrombin complex. ** $P<0.01$.
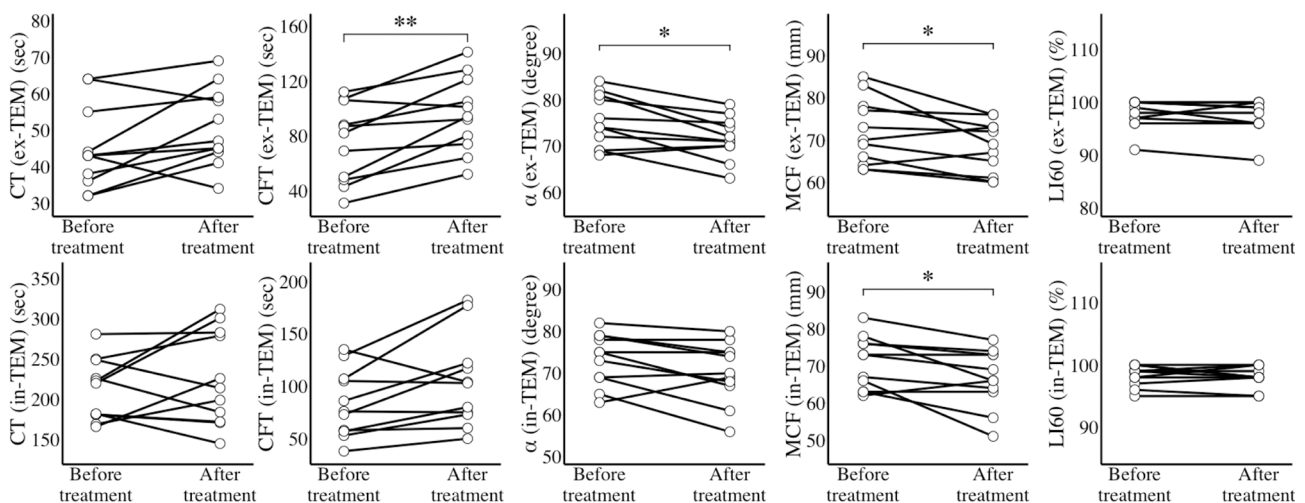
Fig. 3. Changes in coagulation and fibrinolysis parameters measured by rotational thromboelastometry in dogs with protein-losing enteropathy (responders) after treatment. Asterisks indicate significant difference between the values before and after treatment. CT, clotting time; CFT, clot formation time; α, α angle; MCF, maximum clot firmness; LI60, lysis index 60. * $P<0.05$, ** $P<0.01$.

Table 2. Comparison of the clinical, hematological, and conventional coagulation parameters between baseline and after treatment in responders and non-responders based on plasma albumin concentration

Variables	Unit	Reference range	Responders (n=11)			Non-responders (n=3)	
			Before treatment	After treatment	P value	Before treatment	After treatment
ALB	g/dl	2.6–4.0	1.7 (1.1–2.5)	2.8 (1.8–3.6)	0.001	1.7 (1.4–2.2)	1.7 (1.1–2.1)
CIBDAI	-	0–3	4 (1–11)	0 (0–5)	0.016	2 (1–5)	3 (0–4)
CCECAI	-	0–3	8 (1–11)	0 (0–10)	0.009	2 (1–10)	3 (1–10)
Hematocrit	%	37.3–61.7	41.9 (29.8–56.3)	45 (25–53.7)	0.374	47.5 (37–52.9)	41.3 (35.6–49.4)
White blood cell	$10^3/\mu l$	5.05–16.76	14.8 (4.1–24.6)	9.1 (4.9–38.9)	0.067	17.1 (13.9–24.6)	18 (16.2–38.9)
Platelet	$10^3/\mu l$	148–484	410 (277–926)	361 (197–1,058)	0.168	868 (411–926)	615 (360–1,058)
PT	sec	6.8–8.6	7.1 (6.7–7.9)	7.2 (6.8–7.6)	0.477	7.8 (7.4–7.9)	7.1 (7.1–7.6)
aPTT	sec	13.1–26.9	15.7 (7.8–21.9)	14.9 (9.3–23.8)	1.000	19 (18.7–21.9)	19.7 (12.9–23.8)
Fibrinogen	mg/dl	88–336	256 (150–508)	290 (192–417)	0.831	309 (221–412)	272 (200–302)
TAT	ng/ml	<0.20	0.1 (0.06–0.411)	0.1 (0.071–0.943)	0.638	0.2 (0.088–0.411)	0.1 (0.076–0.4)
AT	%	116–161	82 (57–102)	96 (59–140)	0.142	64 (54–82)	84 (77–89)
D-dimer	µg/ml	<1.0	1.1 (0.2–2.77)	0.5 (0.2–2.12)	0.185	1.4 (0.89–1.46)	0.5 (0.42–1.98)

Data are presented as median and range in the parentheses. ALB, albumin; CIBDAI, canine inflammatory bowel disease activity index; CCECAI, canine chronic enteropathy clinical activity index; PT, prothrombin time; aPTT, activated partial thromboplastin time; TAT, thrombin-antithrombin complex; AT, antithrombin.

Table 3. Comparison of the coagulation and fibrinolytic parameters measured by rotational thromboelastometry between before and after treatment in responders and non-responders judged from the change of plasma albumin concentration

Variables	Unit	Control (n=15)	Responders (n=11)			Non-responders (n=3)	
			Before treatment	After treatment	P value	Before treatment	After treatment
CT (ex-TEM)	sec	61 (39–103)	43 (32–64)	47 (34–69)	0.075	41 (36–57)	47 (37–66)
CFT (ex-TEM)	sec	126 (46–213)	82 (31–112)	94 (52–141)	0.007	65 (44–90)	60 (43–63)
α (ex-TEM)	degree	65 (57–82)	74 (68–84)	71 (63–81)	0.018	80 (72–81)	78 (77–81)
MCF (ex-TEM)	mm	59 (48–75)	70 (63–85)	69 (60–76)	0.045	69 (68–75)	72 (71–75)
LI60 (ex-TEM)	%	98 (65–100)	98 (91–100)	98 (89–100)	0.339	89 (77–100)	84 (79–99)
CT (in-TEM)	sec	242 (198–381)	220 (166–281)	214 (145–312)	0.577	203 (186–236)	213 (174–254)
CFT (in-TEM)	sec	157 (65–239)	76 (38–135)	103 (50–182)	0.050	61 (54–86)	65 (44–89)
α (in-TEM)	degree	60 (52–77)	75 (63–82)	70 (56–81)	0.075	77 (73–79)	77 (72–81)
MCF (in-TEM)	mm	57 (49–71)	73 (62–83)	66 (51–77)	0.038	69 (68–73)	72 (70–75)
LI60 (in-TEM)	%	99 (94–100)	99 (95–100)	98 (95–100)	0.665	100 (96–100)	100 (92–100)

Parameters measured by rotational thromboelastometry in clinically healthy dogs (Control) are also shown. Data are presented as median and range in the parentheses. CT, clotting time; CFT, clot formation time; α, α angle; MCF, maximum clot firmness; LI60, lysis index 60.

concentration in the responder dogs before treatment was not markedly elevated and CT imaging evaluated in 5 dogs did not reveal thrombus formation, lack of the significant change of D-dimer value was conceivable reason. The TAT which is formed rapidly after thrombin production is a direct marker for activation of coagulation cascade, and theoretically similar to the CT, CFT and α measured by ROTEM [19, 26, 34]. The value of TAT was relatively low (median 0.1 ng/ml, range 0.06–0.411 ng/ml) in dogs with PLE before treatment, therefore, we could not detect the significant decrease of TAT after treatment. The conventional coagulation tests individually evaluate each parameter, in contrast, the ROTEM analysis comprehensively evaluate the coagulability of whole blood. We assume that several factors contribute to the hypercoagulability in dogs with PLE, therefore, no significant change was observed in conventional coagulation tests. The inflammatory or neoplastic disease can cause hypercoagulability in dogs [21, 25], and we assume that improvement of intestinal inflammation and gastrointestinal lymphoma contributed to amelioration of hypercoagulability in dogs with PLE after treatment.

The significant increase of CFT and decrease of α were observed only in ex-TEM profile. The ex-TEM and in-TEM evaluate extrinsic and intrinsic pathway respectively. The discrepancies of the results in CFT and α between ex-TEM and in-TEM profile suggests that the overactivation of extrinsic pathway improved after treatment of PLE in dogs. The extrinsic pathway is activated by tissue factor, whereas the intrinsic pathway is activated by the contact activation of plasma coagulation factors [15, 24]. The expression of tissue factor is increased by inflammatory cytokines [40]. We assume that the improvement of intestinal inflammation and gastrointestinal lymphoma after treatment decreased expression of tissue factor, and finally resulted in the improvement of hypercoagulability measured in ex-TEM.

The plasma AT activity was below the reference range in all of the dogs with PLE before treatment (14/14; 100%) in this study. However, there was no significant increase in plasma AT activity after treatment even in the treatment responders judged from the increase of plasma ALB concentration. This result does not support our initial hypothesis that plasma AT activity increases when plasma ALB level increases after treatment. Previous reports indicated that the administration of glucocorticoids decreased

plasma AT activity in dogs [36], although the reasons for these changes have not yet been understood. We assume that the amount of enteric loss of AT reduced after treatment. However, the effect of the glucocorticoid, which is used for the treatment of PLE conceivably influenced the reduction of AT activity. As an overall result, there was no significant difference in plasma AT activity between before and after treatment even in the responders.

In our study, dogs that had been already treated with glucocorticoids at the time of diagnosis were shown to be in hypercoagulable state judged from the MCF values (in in-TEM and ex-TEM) obtained by ROTEM. This result was consistent with a previous report described the hypercoagulability induced by glucocorticoid administration in dogs detected by TEG [37]. In addition, although most of the dogs with PLE were treated with glucocorticoids, the coagulation parameters improved after treatment in responders judged from the plasma ALB concentration. Glucocorticoids can cause hypercoagulability and decrease of AT activity in dogs [36, 37], on the other hand, chronic enteritis and gastrointestinal lymphoma can be ameliorated by glucocorticoids [7, 12, 29, 39]. Moreover, L-asparaginase is known to decrease AT activity in dogs [35]. A human study suggests that L-asparaginase decreases the plasma levels of coagulation and anti-coagulation factors including fibrinogen, AT and factor IX and X [28]. The results of thromboelastgrams suggest the tendency for hypercoagulability after administration of L-asparaginase in human medicine [28]. Based on these studies, glucocorticoids and L-asparaginase can cause both aggravation and amelioration of hypercoagulability in dogs with PLE. The results obtained in this study suggests that hypercoagulability indicated by ROTEM improved when the plasma ALB concentration increased even if glucocorticoids and L-asparaginase were medicated.

The dogs with PLE were shown to be hypercoagulable in terms of ROTEM parameters including CT, CFT, α and MCF (both in-TEM and ex-TEM) in this study. The results indicated by ROTEM in this study corresponded to the data obtained by TEG in a previous report [14]. On the other hand, the hypercoagulability indicated by ROTEM was significantly ameliorated in treatment-responsive dogs judged from the change of plasma ALB concentration in the present study, although improvement of the TEG parameters was not found after treatment in the previous report [14]. There are several possibilities for this discrepancy. First, we classified dogs with PLE into responders and non-responders unlike the previous report. Second, the median duration of treatment for PLE in the present study was 45 days, longer than 4–24 days in the previous report [14]. Third, we used ROTEM with stimulation of intrinsic (in-TEM) or extrinsic (ex-TEM) pathway, in contrast to the unactivated TEG in the previous report [14]. We assume these differences contributed to the discrepancy of the results between the present study and the previous study [14].

This study had several limitations. Half of the dogs that were evaluated for the coagulation parameters had been already treated with prednisolone before their enrollment to this study, which could influence to the coagulation and fibrinolysis parameters [36, 37]. In addition, the duration of therapy was not standardized. Moreover, the dogs included in the present study has various underlying diseases of PLE including chronic enteritis and small- and large-cell gastrointestinal lymphomas. The pathophysiology or treatment are different among underlying disease, therefore, further analysis with standardized underlying diseases and treatment are required. It's possible that the drugs used for the treatment of PLE such as chlorambucil affected coagulation status in the present study. The small sample size was also one of the limitations of the present study, in particular regarding the dogs that developed thromboembolism.

In summary, hypercoagulability indicated by ROTEM was found in dogs with PLE and it was ameliorated in the treatment responsive dogs judged from the change of plasma ALB concentration. Further study is warranted for the prediction of thromboembolism in dogs suffered from diseases at risk of thromboembolism.

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