

Standard Article

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Hemostatic Findings in Ascitic Fluid: A Cross-Sectional Study in 70 Dogs

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Background: Ascitic fluids of horses and humans have fibrinolytic activity, independent of the underlying mechanism of fluid formation.

Objective: To determine whether coagulation and fibrinogenolytic/fibrinolytic activity (ie, low fibrinogen and increased fibrin–fibrinogen degradation products [FDPs], D-dimer, or both) occur in all types of ascitic fluid in dogs.

Animals: A total of 70 client-owned dogs with ascites.

Methods: In this cross-sectional study, dogs were categorized based on the pathophysiology of fluid formation into 4 groups: transudates due to decreased osmotic pressure, transudates due to increased hydrostatic pressure, exudates, and hemorrhagic ascites. Fibrinogen, FDPs, and D-dimer concentrations were measured and then compared in both ascitic fluid and plasma.

Results: Ten dogs had transudates due to decreased colloid osmotic pressure, 18 had transudates due to increased hydrostatic pressure, 13 had exudates, and 29 had hemorrhagic ascites. Ascitic fibrinogen concentrations (n = 70) were significantly lower (median = 59 mg/dL; range: 59–122 mg/dL) than those in the plasma (median = 168 mg/dL, range: 59–879 mg/dL; $P < .0001$). Ascitic FDPs concentrations (n = 70) were significantly higher ($<5 \mu\text{g/mL}$: 3/70 dogs, ≥ 5 to $<20 \mu\text{g/mL}$: 11/70 dogs, $\geq 20 \mu\text{g/mL}$: 56/70 dogs) than those in the plasma ($<5 \mu\text{g/mL}$: 17/70 dogs, ≥ 5 to $<20 \mu\text{g/mL}$: 28/70 dogs, $\geq 20 \mu\text{g/mL}$: 25/70 dogs; $P < .0001$). Ascitic D-dimer concentrations (n = 70) were significantly higher (median = 3.98 $\mu\text{g/mL}$, range: 0.02–9.19) than those in the plasma (median = 0.11 $\mu\text{g/mL}$, range: 0.01–4.08; $P < .0001$). Analysis of the data for each of the 4 different types of ascites showed similar results to those of all the data analyzed together.

Conclusions and Clinical Importance: Ascitic fluid of dogs has evidence of coagulation activation and fibrinogenolytic/fibrinolytic activity and that this phenomenon occurs independent of the underlying mechanism that leads to the formation of ascites.

Key words: Ascites; Canine; Fibrinogenolysis; Fibrinolysis.

Ascites is the abnormal accumulation of fluid in the peritoneal cavity, which can be classified according to its pathophysiology of formation in exudate, transudate, and hemorrhagic ascites.¹ Exudates are considered effusions that form secondary to increased permeability of the peritoneal surface affected by inflammatory processes or neoplasia. Causes of exudative ascites in animals are pancreatitis, bile peritonitis, malignant disease involving the peritoneum, and septic peritonitis.² Transudates are the result of systemic conditions altering

Abbreviations:

CHF	congestive heart failure
COP	colloid osmotic pressure
FDPs	fibrin–fibrinogen degradation products
PHF	primary hyperfibrin(nogen)olysis
TNCC	total nucleated cell count
TP	total protein

Starling forces and form across a normal peritoneal surface.^{1,3,4}

Causes of transudative ascites in animals include diseases that cause increased venous hydrostatic pressure with portal hypertension (eg, congestive heart failure [CHF], liver diseases) or decreased colloid osmotic pressure (COP) (eg, protein losing enteropathy, protein losing nephropathy, or severe hepatic failure).² Hemorrhagic ascites occurs secondary to the pathological accumulation of blood in the peritoneal cavity. In animals, hemorrhagic ascites occurs secondary to trauma, spontaneous ruptures of pathological organs, and coagulopathy.²

With the exception of the hemorrhagic ascites, in which the abdominal fluid accumulates due to rupture of blood vessels or secondary to clotting disorders, all the other ascitic fluids originate secondary to exudation or ultrafiltration of plasma. All ascitic fluid contains proteins involved in coagulation in an environment where the actions of these proteins are not well regulated due to the lack of other constituents of the hemostatic system (eg, platelets, vascular endothelium). In 3 human studies with different panels of coagulation assays, all the ascitic fluids evaluated were shown to

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The study was conducted at the San Marco San Marco Veterinary Clinic, Via Sorio 114/c, 35141 Padova, Italy.

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have fibrinolytic activity, independent of the underlying mechanism of formation.⁵⁻⁷ In 2 of these studies, patients with ascites secondary to liver cirrhosis were enrolled and the findings suggested that the inherently fibrinolytic activity of the ascites may contribute to the systemic hyperfibrinolytic state typically seen in advanced liver disease.^{6,7} Also horses with any type of pathological abdominal effusion have a significantly higher ascitic D-dimer concentration than plasma concentration, suggesting there is fibrinolytic activity within the effusion.⁸

The objective of the cross-sectional study reported here was to determine whether coagulation and fibrinogenolytic/fibrinolytic activity (ie, low fibrinogen and increased fibrin–fibrinogen degradation products [FDPs] or D-dimer, or low fibrinogen and increased FDPs and D-dimer) occurs in any type of canine ascitic fluid.

Materials and Methods

Animals

In this study, 219 dogs with abdominal effusion of any origin, as confirmed by abdominal ultrasonography, were studied. The dogs consecutively presented to the San Marco Veterinary Clinic from September 2011 to January 2013. In 81 of these dogs, ascitic fluid collection was performed at the time of presentation, and concurrently (± 2 hours), a cephalic (for medium or large size dogs) or (for small size dogs) jugular venous blood sample was taken for laboratory investigation. Only dogs, in which the pathophysiologic cause for the abdominal effusion formation was established and there was a complete medical record, including history and results of physical examination, CBC (including blood smear examination), serum biochemistry analysis, coagulation profile analysis, urinalysis, ascitic fluid analysis, and additional diagnostic tests necessary to determine a diagnosis, were included in the study. Dogs were excluded from further analysis if they had a concomitant pleural effusion at the time of presentation to keep the studied population as homogeneous as possible. Dogs were also excluded from further analysis if they had received treatment with plasma, plasma derivate or anticoagulant treatment/intoxication within 30 days before study enrollment.

Ascitic Fluid Classification

Abdominal effusions were classified based on the pathophysiology of its formation. According to Starling's law,⁹ transudates were the ascites resulting from decreased COP (group 1) or increased hydrostatic pressure (group 2), and exudates were the ascites resulting from increased vascular permeability (group 3). Finally, the ascitic fluids resulting from blood vessel rupture were classified as hemorrhagic effusions (group 4). The diagnosis of the disease that caused the ascites was used as the gold standard for establishing the pathophysiology of abdominal fluid formation.

Transudates resulting from decreased COP included the effusions from dogs with hypoproteinemia due to protein losing enteropathy, protein losing nephropathy, or severe hepatic failure. Hypoproteinemia, and not hypoalbuminemia, was used as an aid for the indirect identification of decreased COP because in sick human patients a poor correlation exists between COP and serum albumin concentration,¹⁰ and in healthy dogs COP correlates better with serum total protein (TP).^{11,12} Transudates resulting from increased hydrostatic pressure included the effusions from dogs with right-sided CHF or other diseases that caused portal hypertension. Exudates were the effusions from dogs with neoplasia that

directly involved the abdominal wall or the intra-abdominal organs' serosal surfaces, septic and nonseptic peritonitis, or bile peritonitis. Hemorrhagic ascites were the effusions resulting from iatrogenic, traumatic, or spontaneous intra-abdominal bleeding.

Ascitic Fluid Fibrinogen, FDPs, D-Dimer, TP, Hematocrit, and Total Nucleated Cell Count (TNCC) Analysis

The fibrinogen, FDPs, and D-dimer concentrations were measured in all the ascitic fluids. A whole ascitic sample was collected from each dog via ultrasonographic-guided abdominocentesis. Ascitic samples were immediately placed in plastic tubes containing K₃-EDTA for the determination of hematocrit and TNCC, plain tubes for the determination of TP (with an automated chemistry analyzer),^a and plastic tubes containing 3.2% sodium citrate (final ratio of volume of anticoagulant to volume of ascitic fluid, 1 : 9)^b for measurement of the coagulation variables. Tubes containing sodium citrate were centrifuged at 1,500 \times g for 5 minutes. Then, the supernatant of the ascitic fluid was harvested, and fibrinogen, FDPs, and D-dimer concentrations were determined within 1 hour after ascitic sample collection. A board certified clinical pathologist (MC) performed the cytological examinations of all of the ascitic fluids.

Plasma Fibrinogen, FDPs, and D-Dimer Analysis

Plasma concentrations of fibrinogen, FDPs, and D-dimer were determined in all dogs. A whole blood sample (3.5 mL) was collected from each dog via cephalic (for medium or large size dogs) or jugular venipuncture (for small size dogs). Blood samples were immediately placed in plastic tubes containing 3.2% sodium citrate (final ratio of volume of anticoagulant to volume of blood, 1 : 9)^b for measurement of all the coagulation variables. Tubes containing sodium citrate were centrifuged at 1,500 \times g for 5 minutes. Then, the plasma was harvested, and a coagulation profile analysis was performed within 1 hour after blood sample collection. Fibrinogen concentrations were determined via quantitative assays^c with an automated analyzer.^d The detection limit for fibrinogen concentration was 60 mg/dL; if a value was below the detection limit, the value was entered into the data sheet as 59 mg/dL for the statistical analysis. The plasma concentrations of FDPs were determined with a semiquantitative plasma latex agglutination kit^e that was validated^{13,14} for use with canine blood. Plasma D-dimer concentrations were determined with a validated¹⁴ immunoturbidimetric quantitative assay^e with an automated chemistry analyzer.^a

Statistical Analysis

Normality of data was assessed with the Kolmogorov–Smirnov test. Fibrinogen, FDPs, and D-dimer concentrations were compared between ascitic fluid and venous blood via the Wilcoxon signed-rank test (fibrinogen and D-dimer), and Pearson's chi-square test (FDPs). The same analysis by the same statistical tests was applied when the data were analyzed within the 4 groups. Finally, the correlation between ascitic TP and ascitic D-dimer was calculated by rank correlation by means of the Spearman ρ coefficient. For all statistical analyses, values of $P < .05$ were considered significant.

Results

Animals and Ascitic Fluid Classification

Of the 81 dogs from which ascitic fluid and venous blood samples were taken at the time of presentation, 11 dogs (one due to rodenticide exposure, 8 with

concurrent pleural effusion, and 2 due to a lack of a clear pathophysiological cause for the abdominal fluid formation) were excluded. The remaining 70 dogs were included in the study. There were 49 males (44 sexually intact and 5 neutered) and 21 females (13 sexually intact and 8 spayed). The mean age of these dogs was 8.6 ± 3.4 years (range, 1–15 years). Eighteen of the dogs were crossbred, and 52 were purebred (13 German shepherds, 5 Cane Corsos, 5 Labrador retrievers, 3 boxers, 3 cocker spaniels, 3 Yorkshire terriers, 2 Maltese, 2 Siberian huskies, and 1 of each of the following 16 breeds: American Staffordshire terrier, beagle, Bernese mountain dog, bichon frise, border collie, Doberman, fox terrier, great Dane, greyhound, leonberger, German pinscher, rottweiler, shar pei, toy poodle, West Highland white terrier, and whippet). The ascitic fluids were classified as follows: 10 as transudates resulting from decreased COP, 18 as transudates resulting from increased hydrostatic pressure, 13 as exudates, and 29 as hemorrhagic. The underlying causes for ascites formation are summarized in Table 1.

Ascitic Fluid TP, Hematocrit, and TNCC Analysis

The hematological and ascitic fluid characteristics of the dogs included in the study are summarized in Table 2. As expected, all transudates due to decreased

COP had serum TP <4 g/dL, which is the cutoff value under which the occurrence of a vascular fluid outflow due to decreased COP has previously been suggested.¹⁵ All the increased hydrostatic pressure transudates had serum TP >4 g/dL and ascitic TP similar to the exudates, but low ascitic TNCC. The exudative ascites had the highest fluid TNCC count. Finally, the hemorrhagic ascites had a similar ascitic and peripheral TP and hematocrit, and all the ascitic hematocrit were at least >50% of the peripheral hematocrit, whereas in all the other types of effusions, the ascitic hematocrit never reached 25% of the peripheral hematocrit (data not shown).

Ascitic Fluid and Plasma Fibrinogen, FDPs, and D-Dimer Analysis

Ascitic fibrinogen concentrations (median = 59 mg/dL; range: 59–122 mg/dL) were significantly ($P < .0001$) lower than those in the plasma (median = 168 mg/dL; range: 59–879 mg/dL; Fig 1). In each dog, when the ascitic fibrinogen concentration was compared to its corresponding plasma value, the ascitic fibrinogen concentration was lower than the plasma sample in all but 1 dog, and equal in the remaining case. Ascitic fibrinogen concentrations were lower than the plasma reference interval (152–284 mg/dL) in all 70 samples, and in

Table 1. Causes of ascites in 70 dogs.

Group 1 (n = 10)	Group 2 (n = 18)	Group 3 (n = 13)	Group 4 (n = 29)
PLE (5)	Right-sided CHF (14):	Neoplasia directly involving the abdominal wall	Hemangiosarcoma (21)
PLN (1)	– Neoplastic PE (5)	or the intra-abdominal organ serosal surfaces (5)	Other malignancies (4)
Concurrent PLN	– Idiopathic PE (3)	Nonseptic peritonitis (3)	Benign tumor (1)
and PLE (2)	– Myocardial, valvular, and/or	Septic peritonitis (3)	Necrotizing hepatitis (1)
Hepatic failure (2)	arrhythmogenic diseases (6)	Bile peritonitis (2)	Iatrogenic (1)
	Other diseases causing portal		Traumatic (1)
	hypertension (4)		

Group 1: transudates due to decreased colloid osmotic pressure; Group 2: transudates due to increased hydrostatic pressure; Group 3: exudates; Group 4: hemorrhagic ascites; CHF, congestive heart failure; PLE, protein losing enteropathy; PLN, protein losing nephropathy; PE, pericardial effusion.

Table 2. Hematological and ascitic fluid characteristics in the 4 groups of dogs with ascites included in the study.

	Group 1 (n = 10)	Group 2 (n = 18)	Group 3 (n = 13)	Group 4 (n = 29)
Blood Hct (%)	41.5 (19.2–69.8)	40.5 (14.0–67.0)	43.8 (24.0–59.4)	28.9 (13.4–53.2)
Median (range)				
Serum TP (g/dL)	3.1 (2.2–3.9)	5.0 (4.2–6.2)	6.2 (4.4–7.5)	5.8 (4.2–7.7)
Median (range)				
Serum Albumin (g/dL)	1.3 (0.9–1.4)	2.5 (1.4–2.8)	2.5 (1.8–3.1)	2.8 (1.5–3.8)
Median (range)				
Ascitic Hct (%)	0.0 (0.0–0.1)	0.1 (0.0–2.2)	0.7 (0.2–9.4)	29.8 (7.9–45.8)
Median (range)				
Ascitic TP (g/dL)	0.2 (0.1–0.4)	3.5 (0.1–5.4)	3.7 (1.8–5.1)	5.1 (3.1–8.0)
Median (range)				
Ascitic TNCC (10 ³ μL)	0.19 (0.02–0.86)	0.33 (0.01– 1.17)	28.06 (2.30–173.55)	11.12 (3.10–33.80)
Median (range)				

Group 1: transudates due to decreased colloid osmotic pressure; Group 2: transudates due to increased hydrostatic pressure; Group 3: exudates; Group 4: hemorrhagic ascites; Hct, hematocrit; TP, total protein; TNCC, total nucleated cell count.

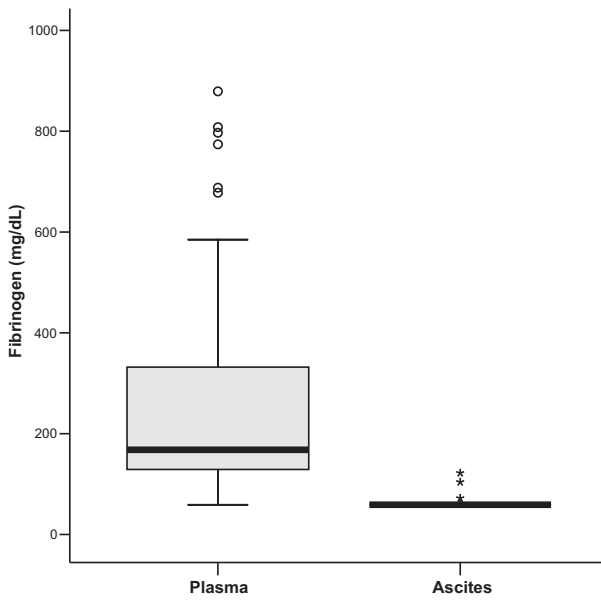


Fig 1. Box-and-whisker plots of plasma and ascitic fibrinogen concentrations in samples obtained from all the 70 dogs included in the study. Ascitic fibrinogen concentrations (median = 59 mg/dL; range: 59–60 mg/dL) were significantly ($P < .0001$) lower than plasma fibrinogen concentrations (median = 168 mg/dL; range: 59–879 mg/dL). For each box, the horizontal line represents the median value and lower, and upper boundaries represent 25th and 75th percentiles, respectively. Whiskers represent the most extreme observation that are not outliers. White circles represent outlier values (more than 1.5 interquartile range away from the closest end of the box), and black stars represent extreme outlier values (more than 3 interquartile range away from the closest end of the box).

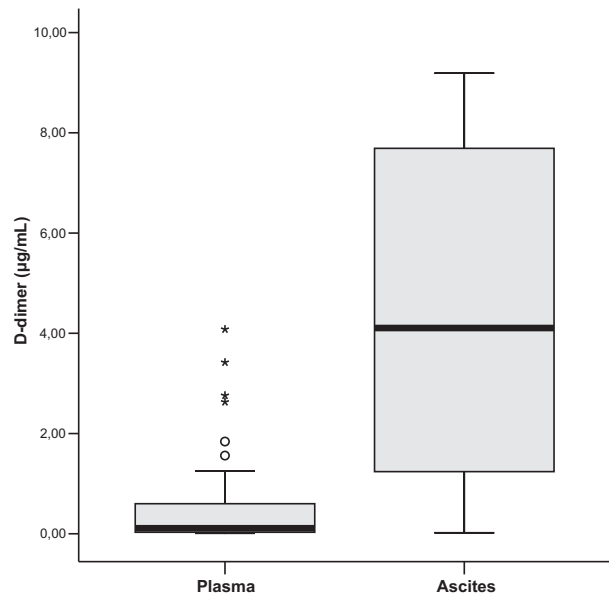


Fig 2. Box-and-whisker plots of plasma and ascitic D-dimer concentrations in samples obtained from all the 70 dogs included in the study. Ascitic D-dimer concentrations were significantly ($P < .0001$) higher (median: 3.98 µg/mL; range: 0.02–9.19) than plasma D-dimer concentrations (median: 0.11 µg/mL; range: 0.01–4.08). For each box, the horizontal line represents the median value, and lower and upper boundaries represent 25th and 75th percentiles, respectively. Whiskers represent the most extreme observation that are not outliers. White circles represent outlier values (more than 1.5 interquartile range away from the closest end of the box), and black stars represent extreme outlier values (more than 3 interquartile range away from the closest end of the box).

65 of 70 ascitic fluids, the fibrinogen concentrations were under the limit of detection of the instrument (ie, <60 mg/dL). In the 5 samples with fibrinogen concentrations above the lower limit of detection, 2 measured at 60 mg/dL, and the other 3 measured 72, 104, and 122 mg/dL.

The ascites and plasma concentrations of FDPs are summarized in Table 3 for all 70 dogs. On average, the concentrations of FDPs in the ascitic fluid were significantly ($P < .0001$) higher than those in the plasma. In each dog, when the ascitic concentration of FDPs was compared to its corresponding plasma value, the ascitic concentration of FDPs was higher than the plasma sample in 36 cases, equal in 33 cases, and lower in 1 case. Ascitic concentrations of FDPs were higher than the plasma reference interval (<5 µg/mL) in 67 of the 70 ascitic fluids.

Table 3. Comparison of ascitic and plasma concentrations of fibrin–fibrinogen degradation products (FDPs)

FDPs (µg/mL)	Ascites (n [%] of Dogs)	Plasma (n [%] of Dogs)
<5	3 (4.3)	17 (24.3)
≥5 to <20	10 (14.3)	28 (40.0)
≥20	57 (81.4)	25 (35.7)

Ascitic D-dimer concentrations were significantly ($P < .0001$) higher (median = 3.98 µg/mL; range: 0.02–9.19) than they were in the plasma (median = 0.11 µg/mL; range: 0.01–4.08; Fig 2). In each dog, when the ascitic D-dimer concentration was compared to its corresponding plasma value, the ascitic D-dimer concentration was higher than the plasma sample concentration in 65 cases, lower in 4 cases, and equal in 1 case. Ascitic concentrations of D-dimer were higher than the plasma reference interval (0.01–0.34 µg/mL) in 66 of the 70 ascitic fluids.

In conclusion, all the ascitic fluids had low or a non-detectable fibrinogen concentrations and at least 1 fibrin/fibrinogenolytic marker above the reference interval of what is considered normal in a plasma sample.

Analysis of the data divided into the 4 groups (ie, decreased COP and increased hydrostatic pressure transudates, exudates, and hemorrhagic ascites) showed similar results as when all the data were analyzed together. In all 4 groups, the median ascitic fibrinogen concentrations were significantly lower than were the respective median plasma concentrations, and the median ascitic D-dimer concentrations were significantly higher than their respective median plasma concentrations (Table 4). Also, the concentrations of FDPs in the ascites were, on average, higher than their respective

Table 4. Concentrations of fibrinogen, D-dimer, and FDPs in ascites and plasma for 70 dogs with ascites.

Parameter		Ascites	Plasma	P Value
Group 1 (n = 10)	Fibrinogen (mg/dL)	59 (59–59)	344 (79–774)	.005
	Median (range)			
	D-dimer (µg/mL)	1.68 (0.44–5.65)	0.05 (0.01–0.72)	.005
	Median (range)			
Group 2 (n = 18)	Fibrinogen (mg/dL)	59 (59–72)	164 (60–678)	<.0001
	Median (range)			
	D-dimer (µg/mL)	2.55 (0.29–9.19)	0.05 (0.01–0.78)	<.0001
	Median (range)			
Group 3 (n = 13)	Fibrinogen (mg/dL)	59 (59–122)	327 (59–879)	.002
	Median (range)			
	D-dimer (µg/mL)	6.79 (0.02–8.71)	0.15 (0.01–2.76)	.006
	Median (range)			
Group 4 (n = 29)	Fibrinogen (mg/dL)	59 (59–59)	157 (60–459)	<.0001
	Median (range)			
	D-dimer (µg/mL)	5.86 (0.10–9.05)	0.26 (0.01–4.08)	<.0001
	Median (range)			
FDPs (µg/mL)	<5	n = 2	n = 7	
	≥5 to <20	n = 5	n = 2	.079
	≥20	n = 3	n = 1	
FDPs (µg/mL)	<5	n = 1	n = 1	
	≥5 to <20	n = 2	n = 14	.0002
	≥20	n = 15	n = 3	
FDPs (µg/mL)	<5	n = 0	n = 3	
	≥5 to <20	n = 0	n = 2	.045
	≥20	n = 13	n = 8	
FDPs (µg/mL)	<5	n = 0	n = 6	
	≥5 to <20	n = 3	n = 10	.0008
	≥20	n = 26	n = 13	

Group 1: transudates due to decreased colloid osmotic pressure; Group 2: transudates due to increased hydrostatic pressure; Group 3: exudates; Group 4: hemorrhagic ascites; FDPs, fibrin–fibrinogen degradation products.

plasma concentrations in all the 4 groups. However, this difference did not reach statistical significance in group 1 (decreased COP) (Table 4).

Finally, there was a significant ($P = .001$) weak positive correlation ($\rho = 0.39$) between ascitic TP and ascitic D-dimer.

Discussion

In this cross-sectional study, we investigated whether coagulation and fibrinogenolytic/fibrinolytic activity occurs in any type of canine ascitic fluid. The results show that (1) in the ascitic fluid of dogs, there is evidence of coagulation activation and fibrinolysis in almost all cases, and (2) this phenomenon occurs independent of the underlying mechanism that leads to ascites formation.

The first statement is supported by the finding that fibrinogen concentrations in dogs are significantly lower (and most of the time undetectable) in the ascitic fluid, compared to plasma, whereas FDPs and D-dimer ascitic concentrations are significantly higher than those in the plasma. All together, these results would suggest that fibrinogen, upon entrance into the abdominal cavity, is transformed into cross-linked fibrin and then lysed to form D-dimer. The increased FDPs would support this finding, but also suggests that some fibrinogen, fibrin monomer or polymers may get lysed even before being

transformed in cross-linked fibrin. The same conclusion has been reported in human medicine.^{5–7}

To assess whether the fibrinogenolytic/fibrinolytic activity of the ascitic fluid depends on the type of abdominal effusions, the data were also analyzed categorizing the abdominal fluids according to their pathophysiology of formation (ie, transudates due to decreased COP, transudates due to increased hydrostatic pressure, exudates, and hemorrhagic ascites). In all of the 4 groups, ascitic fibrinogen concentrations were significantly lower than were the plasma fibrinogen concentrations, whereas the D-dimer ascitic concentrations were significantly higher than the plasma D-dimer concentrations. This further suggests that the fibrinogen, with the aid of the proteins involved in coagulation, is transformed to cross-linked fibrin upon entrance into the abdominal cavity and is then lysed to FDPs and D-dimer, regardless of the pathophysiology of fluid formation. Also, the concentrations of the FDPs in the ascites were higher than were their respective plasma concentrations in all of the 4 groups. However, this difference did not reach statistical significance in group 1. While it is possible that there was really no significant difference between plasma and ascites FDPs' concentrations in group 1, 3 alternative explanations should be also considered. First, group 1 includes a small number of dogs, limiting the statistical power to recognize small differences between concentrations of FDPs in the

ascites and plasma. Second, in the assay that was used concentrations of FDPs are expressed semiquantitatively, limiting the possibility of assessing the magnitude of FDPs' concentrations between ≥ 5 and < 20 and ≥ 20 $\mu\text{g/mL}$ which are FDPs values more frequently present in the ascites rather than the plasma. Third, as shown from the positive correlation between ascitic TP and ascitic D-dimer, it appears that a lower ascitic TP concentration corresponds with a lower ascitic D-dimer. This would also suggest that the fibrinogen (and the other clotting proteins) in the ascites of group 1 was in small concentrations, even before being transformed in cross-linked fibrin and then in D-dimer. Therefore, the lower FDPs and D-dimer in group 1, and the lack of statistical difference between ascitic and plasma FDPs in this type of ascites fluid, more likely represent a decreased fibrin formation due to an originally low fibrinogen concentration rather than a decreased fibrinolytic activity in this fluid. In addition to a possible low fibrinogen concentration, the lower FDPs and D-dimer in group 1 may also be caused by a lower concentration of plasminogen activators, plasmin, or plasmin-like factors in this type of ascitic fluid.

The results of this study, suggesting that the activation of coagulation followed by fibrinolysis occurs in ascitic fluids regardless of the pathophysiology of fluid formation, are in agreement with 1 study in horses,⁸ and 2 studies in humans with ascites.^{6,7} In the study in horses, the D-dimer concentration in the peritoneal fluid was significantly (10 times) higher than its concentration in plasma. This result was considered consistent with peritoneal hyperfibrinolysis by the authors.⁸ In the 2 studies in humans, 25 patients with portal hypertension and ascites secondary to liver cirrhosis were enrolled, in addition to 10 patients with ascites but without liver diseases. As in the dogs of this study, all 35 human patients with ascites had concentration of FDPs and D-dimer in the ascitic fluid that, for the plasma, would be considered quite high.^{6,7} Fibrinogen concentrations were depleted to levels that, for plasma, would be considered quite low.^{6,7} The authors of both studies concluded that any abdominal fluid should be considered inherently fibrinolytic and a possible contributor for the systemic increased fibrinogenolytic/fibrinolytic activity present in the subset of patients with ascites secondary to portal hypertension due to liver cirrhosis.^{6,7}

The hypothesis that ascitic fluid is inherently fibrinolytic is also supported by several observations/pieces of evidence. First, the fluid of virtual cavities, of which ascites is a pathological manifestation, needs to be free of clots to allow for smooth sliding of organs over each other, and therefore, clots need to be rapidly lysed. Second, the hypothesis is supported by the clinical observation that ascitic and pleural effusions rarely form clots in vivo. This observation is confirmed by an experiment in dogs in which the inoculation of blood or of a solution containing fibrinogen and thrombin into the pleural cavity caused the activation of the coagulation system followed by a rapid fibrinogenolysis/fibrinolysis, allowing to the inoculated blood to remain in the large part fluid.¹⁶ These early findings have been later

confirmed by another experimental study in dogs¹⁷ and by clinical studies in humans with hemothorax.^{18,19} Similar results have also been found after the inoculation of blood in the peritoneal cavity of dogs, which remains in the large part fluid unless these dogs were pretreated by continuous infusion with epsilon-aminocaproic acid (an antifibrinolytic drug) before and during the experiment.²⁰ Third, a study of 23 plasma proteins revealed that only fibrinogen and plasminogen had a lower concentration than expected in the ascitic fluid, suggesting an accelerated degradation of these proteins in plasmin and FDPs after the activation of the fibrinolytic system within this fluid.⁵ The activation of the plasminogen in the ascitic fluid is due to the presence of tissue plasminogen activator, urinary plasminogen activator, or both enzymes which can be released (from a preformed storage pool) or leak in the ascitic fluid (following the damage induced by the disease causing the ascites formation) from the mesothelial and submesothelial endothelium cells.^{5,21–27} Finally, in support of the fibrinolytic activity of the ascitic fluid, there is also the evidence of primary hyperfibrinolysis in ascitic human patients treated with palliative peritoneovenous shunts after the reinfusion of their abdominal fluid in the systemic circulation.^{23,26,28–30}

Primary hyperfibrinolysis, also sometimes named primary hyperfibrinogenolysis,^{31,32} is a rare condition that occurs when the generation of plasmin within the general circulation (plasminemia) exceeds the neutralizing capacity of the antiplasmins.³³ In humans, this has been reported in association with acute conditions, such as shock,³¹ surgical procedures,³⁴ liver transplantation,³⁵ acute leukemia,³⁶ or the administration of thrombolytic drugs. It can also be caused by chronic conditions such as neoplasia,³⁷ chronic liver disease,³⁸ or, as said before, after peritoneovenous shunting.^{23,26,28–30} Recently, it has been demonstrated that 90% of dogs with ascites secondary to right-sided CHF have abnormalities in their coagulation tests, suggesting primary hyperfibrinolysis (PHF).³⁹ The prevalence of PHF in dogs with right-sided CHF was significantly higher compared to what found in dogs with left-sided CHF and in sick dogs without cardiac diseases, suggesting a primary role for the ascites in this coagulation disorder.³⁹ The results of this study, which show that ascitic fluids have fibrinolytic activity, may support the hypothesis that the observed PHF described in dogs with ascites secondary to right-sided CHF³⁹ may have possibly been caused by the reabsorption of their ascitic fluid containing plasminogen activator, plasmin, or plasmin-like factors. Whether this is true, or if other mechanisms for the observed PHF are also present, needs to be explored in further studies.

One limitation of this study is that ascitic fibrinogen, FDPs, and D-dimer concentrations were measured with kits that had been validated only in canine plasma samples and not in canine ascitic samples. Nevertheless, the 2 human studies and the 1 study in horses to which this article refers also adopted the same methodology without apparent problems.^{6–8} A second limitation of this study is that the ascitic

concentrations of tissue plasminogen activator, urinary plasminogen activator, and plasmin were not measured. The determination of these enzymes would have strengthened our findings.

In summary, this study shows that ascitic fluid has fibrinogenolytic/fibrinolytic activity, independent of the underlying mechanism of intra-abdominal fluid accumulation. This result, together with the observation that most dogs with ascites secondary to right-sided CHF have coagulation tests that suggest PHF,³⁹ should encourage further studies to ascertain whether or not fibrinolytic ascitic fluid is associated with PHF in dogs.

Footnotes

- ^a Olympus AU 2700, Olympus Diagnostics, Hamburg, Germany
^b 3.2% sodium citrate Vacuette® 3.5 mL, Grenier Bio-One, Kremsmünster, Austria
^c STA Fibrinogen, Diagnostica Stago, Asnières sur Seine, France
^d STA-R Evolution, Diagnostica Stago, Rhoce, Bâsel, Switzerland
^e FDPs plasma, Diagnostica Stago, Asnières sur Seine, France
^f Caldin M, Fulanello T, Lubas G. Sensitivity and specificity of citrated plasma FDPs and D-dimer in the diagnosis of disseminated intravascular coagulation (DIC) in dogs. *J Vet Intern Med* 1998;12:236 (abstract)
^g Tina-quant D-Dimer, Roche Diagnostic GmbH, Mannheim, Germany

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

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