D-dimer concentrations in healthy dogs and dogs with disseminated intravascular coagulation

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Objective—To determine sensitivity and specificity of assays of D-dimer concentrations in dogs with disseminated intravascular coagulation (DIC) and healthy dogs and to compare these results with those of serum and plasma fibrin-fibrinogen degradation product (FDP) assays.

Animals—20 dogs with DIC and 30 healthy dogs.

Procedure—Semi-quantitative and quantitative D-dimer concentrations were determined by use of latex-agglutination and immunoturbidometry, respectively. Fibrin-fibrinogen degradation products were measured by use of latex-agglutination. A reference range for the immunoturbidometric D-dimer concentration assay was established; sensitivity and specificity of the assay were determined at 2 cutoff concentrations (0.30 μg/ml and 0.39 μg/ml).

Results—Reference range for the immunoturbidometric D-dimer concentration assay was 0.08 to 0.39 μg/ml; median concentrations were significantly higher in dogs with DIC than in healthy dogs. Latex-agglutination D-dimer and serum and plasma FDP assays had similar sensitivity (85 to 100%) and specificity (90 to 100%); the immunoturbidometric assay had lower specificity (77%) at the 0.30 μg/ml cutoff and lower sensitivity (65%) at the 0.39 μg/ml cutoff. Sensitivity or specificity of the latex-agglutination D-dimer assay was not significantly improved when interpreted in series or parallel with FDP assays.

Conclusions and Clinical Relevance—Measurement of D-dimer concentrations by latex-agglutination appears to be a sensitive and specific ancillary test for DIC in dogs. Specificity of D-dimer concentrations in dogs with systemic disease other than DIC has not been determined, therefore FDP and D-dimer assays should be performed concurrently as supportive tests for the diagnosis of DIC in dogs. (*Am J Vet Res* 2000;61:393–398)

Disseminated intravascular coagulation (DIC) is a potentially devastating hemostatic defect complicating the clinical course of common diseases such as neoplasia, sepsis, pancreatitis, and heatstroke. Diagnosis of DIC relies on laboratory determination of multiple abnormalities in hemostasis, including thrombocytopenia, prolonged clotting times, low **antithrombin (AT)** activity, and increased concentration of **fibrin-fibrinogen degradation products (FDP)**, together with a high clinical suspicion for DIC.[1,2] The underlying pathogenesis of DIC is widespread activation of coagulation and fibrinolysis with systemic generation of thrombin and plasmin.[1,3,4]

Soluble fibrin is generated from thrombin cleavage of fibrinogen. Thrombin also activates factor XIII that, in the presence of calcium, crosslinks fibrin at 2 sites: γ-chain crosslinks between terminal or D-domains on adjacent fibrin monomers (stabilizing fibrin longitudinally) and α-chain crosslinks between 2 adjacent fibrin polymers (producing a 3-dimensional lattice).

In pathologic states, plasmin cleaves fibrinogen and noncrosslinked (soluble) fibrin, yielding FDP fragments X, Y, D, and E (**Fig 1A**). In physiologic and pathologic states, plasmin cleaves crosslinked fibrin, producing large fragments with molecular weights $> 10^6$ d, called X-oligomers. Continued plasmin cleavage of X-oligomers produces the smallest crosslinked degradation product: fragment E noncovalently bound to 2 D fragments (D-D fragment or D-dimer; **Fig 1B**).[5]

Polyclonal antibodies against FDP (specifically D and E fragments) have been traditionally used in latex-agglutination assays to detect FDP in dogs.[1,2] However, these antibodies cross-react with intact fibrinogen, requiring serum preparation using specialized collection tubes.[6] Latex-agglutination assays for detection of FDP in plasma are currently available. These assays contain monoclonal antibodies that do not cross-react with intact fibrinogen in plasma. Assays using polyclonal or monoclonal antibodies against FDP do not discriminate between products of plasmin degradation of fibrinogen or fibrin.[4,6] In contrast, newer assays using antibodies against degradation products of crosslinked fibrin are more specific measures of fibrinolysis, because they indicate generation of thrombin and plasmin.[6-9] The most commonly used assay for detecting crosslinked fibrin degradation products is the D-dimer assay. The D-dimer epitope is a neoantigen produced by FXIIIa-mediated crosslinking of γ-chains of D domains of 2 adjacent fibrin monomers (Fig 1B). Monoclonal antibodies against this neoantigen recognize D-dimer epitopes within X-oligomers[9,10] and are used to measure D-dimer concentrations in ELISA and immunoturbidometric and latex-agglutination assays.[11,12]

Measurement of both D-dimer and FDP concentrations is recommended for diagnosis of DIC in humans.[7] High D-dimer concentrations are observed in humans with DIC,[7,9,13-16] and D-dimer assays are similarly[7] or more sensitive[13,16] than FDP assays. In 2 pre-

Received Dec 11, 1998.
Accepted Jun 9, 1999.
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Supported by Cornell Unrestricted Alumni Funds.
The authors thank Tara Sparks, Linda Chapman, and Amy Ross for technical assistance.

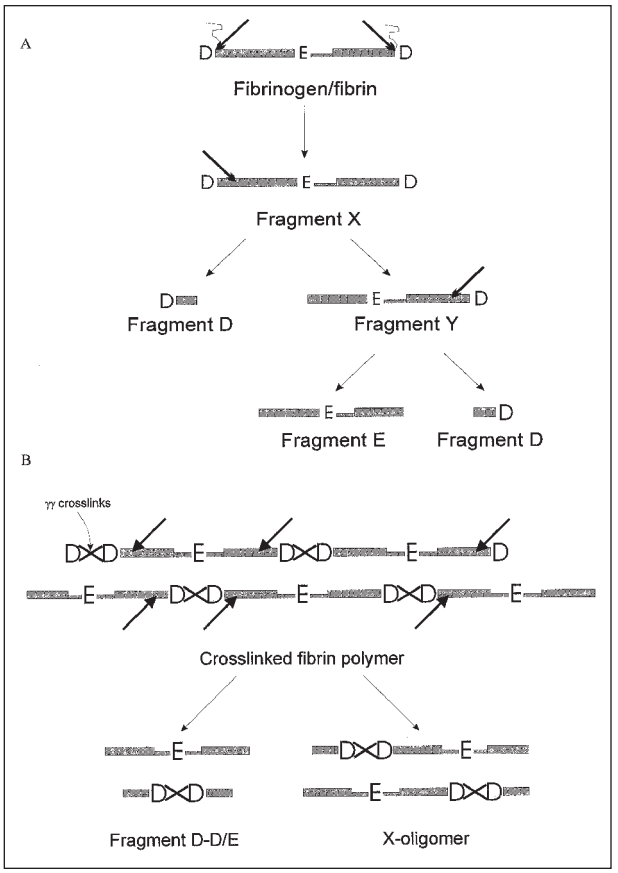

Figure 1—Plasmin cleavage of fibrinogen and noncrosslinked and crosslinked fibrin. A—Cleavage of fibrinogen and noncrosslinked fibrin. Fibrinogen is composed of 2 sets of 3 identical protein chains (Aα, Bβ, and γγ) held together by disulfide bonds, forming a trinodular structure composed of 2 large terminal D domains (carboxy-termini of the protein chains) and a smaller central E domain (amino-termini). Thrombin cleaves the amino-terminal ends of the Aα- (fibrinopeptide A) and Bβ-chains (fibrinopeptide B) to yield noncrosslinked fibrin (illustrated by the short chain between the E and D domains). Plasmin (thick arrows) cleaves the carboxy-terminus of the α-chain in the D domains of fibrinogen and noncrosslinked fibrin (illustrated by curved lines off the D-domains) to yield fragment X. Plasmin then cleaves fragment X between the D and E domains to yield transient fragment Y and fragment D. Further cleavage of fragment Y produces another fragment D and fragment E. B—Cleavage of crosslinked fibrin. Thrombin-activated factor XIII forms γγ bonds at the terminal D domains of 2 adjacent fibrin monomers within the fibrin polymer. This crosslinking produces a neoantigen, called D-dimer. Factor XIIIa also forms αα-crosslinks between 2 adjacent fibrin polymers (not shown). Plasmin degrades α-chain crosslinks between adjacent fibrin protofibrils and cleaves between the D and E domain (arrows) within the fibrin polymer to yield crosslinked high-molecular-weight oligomers (X-oligomers). The terminal degradation product of crosslinked fibrin is the D-D/E fragment that consists of 2 covalently bonded D-domains (D-dimer) bound noncovalently to fragment E.

liminary reports,[a,b] D-dimer concentration (as measured by an immunoturbidometric assay) was a sensitive and specific test for diagnosis of DIC in dogs. To our knowledge, there have been no reports of the use of latex-agglutination D-dimer assays in dogs. The objectives of this study were to determine and compare sensitivity and specificity of latex-agglutination and immunoturbidometric D-dimer assays for diagnosis of DIC in dogs, to compare these results with those from serum and plasma FDP assays, and to determine reproducibility of results of latex-agglutination D-dimer assays for measuring D-dimer concentrations in canine plasma

Materials and Methods

Sample collection—Blood samples were collected from dogs suspected of having DIC, on the basis of clinical criteria. After coagulation testing had been performed, only dogs fulfilling predefined criteria for DIC were included in the study. Clinical criteria were identification of a primary disease process associated with DIC[1] and bleeding from ≥ 2 unrelated sites. Laboratory criteria were thrombocytopenia (platelet count < 179×10^9/L) and ≥ 2 of the following abnormalities: prolonged **prothrombin time (PT)**, prolonged **activated partial thromboplastin time (APTT)**, decreased AT activity, and morphologic changes in erythrocytes in blood smears compatible with fragmentation (ie, acanthocytes, schistocytes, and keratocytes). Results from dogs that fulfilled all criteria for DIC were used to determine sensitivity. Blood samples were collected from 30 clinically healthy veterinary student- and faculty-owned dogs; results from these dogs were used to determine specificity.

Blood samples were collected into tubes containing EDTA for platelet counts and assessment of RBC morphology, specialized collection tubes provided with each kit for measurement of serum FDP concentration, and 3.8% sodium citrate (1:9 citrate:blood) for coagulation testing and measurement of plasma D-dimer and FDP concentrations.

D-dimer assay—D-dimer concentration was measured in citrate plasma by use of latex-agglutination and immunoturbidometry. Plasma was separated as soon as possible after collection and frozen at –25 C. Samples were assayed in batches within 1 month of collection. Latex-agglutination assays were performed by use of a commercial D-dimer kit,[c] according to manufacturer's recommendations. Briefly, undiluted plasma was mixed with a suspension of latex beads coated with a murine monoclonal antibody that reacts with D-dimer in human sera.[17] Agglutination was evaluated after 180 seconds; results were reported as positive or negative. According to the manufacturer, a positive reaction is equivalent to a D-dimer concentration > 0.25 μg/ml. Positive and negative human control sera were included with each assay. We determined reproducibility of the latex-agglutination D-dimer assay in frozen canine plasma samples by measuring D-dimer concentrations in samples from all dogs on a second occasion, after samples had been stored at –25 C for 2 to 12 months. The commercial kit[d] used for these determinations contained the same monoclonal antibody as the original kit,[c] although it was obtained from a different supplier. If D-dimer concentrations in stored frozen samples changed from original values, results were verified by performing the assay concurrently with the original latex-agglutination D-dimer kit.

The immunoturbidometric assay[e] was performed by use of an automated chemistry analyzer.[f] This is an end-point reaction measuring change in turbidity caused by antigen-antibody complexes that are formed after addition of monoclonal antibody-coated latex beads to patient samples containing D-dimer. The D-dimer result is reported in micrograms per milliliter of fibrinogen equivalents, or the amount of fibrinogen initially present that results in the observed concentration of D-dimer. The actual D-dimer concentration is approximately 50% of this value.[7] For determination of intra-assay variation, samples from 5 dogs (range in D-dimer concentrations, 0.16 to 0.64 μg/ml) were assayed 6 times within 1 run. For determination of inter-assay variation, samples from 5 dogs (range in D-dimer concentrations, 0.26 to 0.74 μg/ml) were split into 6 equal aliquots and stored at 4 C. D-dimer concentrations were determined from these aliquots on 6 consecutive days. Furthermore, inter-assay

variability was determined by measuring D-dimer concentrations of 2 human control sera[e] (D-dimer concentration, 0.98 and 4.29 µg/ml) on 6 consecutive days.

Fibrin-fibrinogen degradation product assays—Fibrin-fibrinogen degradation products concentrations in serum and plasma were measured by use of latex-agglutination. Two serum kits were evaluated (TW[g] and SP[h]); the TW kit has traditionally been used for measurement of FDP concentration in dogs[2] and contains antibodies raised in sheep against human D and E fragments. The SP kit had the highest sensitivity of several serum FDP kits[18] and contains antibodies raised in rabbits against human D and E fragments. Each kit provides collection tubes containing *Bothrops atrox* venom and a fibrinolytic inhibitor (soybean trypsin inhibitor or aprotonin) for preparation of serum. Serum was separated from collection tubes after incubation at 20 C (TW) or 37 C (SP) for 30 minutes, then stored at –25 C. Samples were assayed in batches within 1 month of collection. A plasma FDP kit[i] was evaluated as a direct comparison to D-dimer in the same sample (citrate).

The FDP assays were performed according to manufacturers' recommendations. Samples for determination of serum FDP concentration were assayed at dilutions of 1:5 and 1:20. Negative samples (FDP concentration, < 10 µg/ml) lacked agglutination in both dilutions; positive samples agglutinated at the 1:5 dilution (FDP concentration, 10 to 40 µg/ml) or both dilutions (FDP concentration, > 40 µg/ml). Plasma samples were diluted 1:2 and 1:8 and observed for agglutination. Results were similarly reported as negative (FDP concentration, < 5 µg/ml) or positive (FDP concentrations, 5 to 20 µg/ml or > 20 µg/ml).

Laboratory assays for diagnosis of DIC—Platelet counts, assessment for erythrocyte fragmentation, and coagulation testing were performed on samples from dogs suspected of having DIC. Platelet counts were measured from blood samples anticoagulated with EDTA by impedance methods[j] or manually with a hemocytometer. Blood smears stained with Wright's stain were examined for platelet clumps to verify platelet counts. Erythrocytes were examined in blood smears for morphologic evidence of fragmentation. Prothrombin time and APTT were measured in citrate plasma samples, using commercial reagents[k,l] and standard methods.[19] Antithrombin activity was determined in citrate plasma samples by use of a colorimetric method and chromogenic substrate kit[m]; results were reported as a percentage of pooled canine reference plasma (AT activity of 100%).

Statistical analyses—Point estimates with 95% confidence intervals were calculated for sensitivity (ie, number of positive results in 20 dogs with DIC) and specificity (ie, number of negative results in 30 healthy dogs) of latex-agglutination D-dimer and serum and plasma FDP assays. Median immunoturbidometric D-dimer concentrations in dogs with DIC and healthy dogs were compared by use of the rank sum test.[n] Point estimates with 95% confidence intervals for sensitivity and specificity of the immunoturbidometric D-dimer assay were determined for 2 cutoff concentrations. The first cutoff was selected on the basis of a receiver-operator characteristic curve for D-dimer concentrations[20]; the second cutoff was selected on the basis of the upper limit of the reference range, as determined from D-dimer concentrations of 30 healthy dogs. Point estimates and 95% confidence intervals were calculated for sensitivity and specificity of D-dimer and FDP assays performed in parallel (ie, a positive test for DIC was equivalent to a positive result in D-dimer or FDP assays) and series (ie, a positive test for DIC was equivalent to a positive result in D-dimer and FDP assays). Kappa statistics were calculated to test concordance among pairs of tests for positive and negative results. A kappa statistic < 0.4 was considered poor, whereas a kappa statistic > 0.7 was considered good to excellent. Confidence intervals and kappa statistics were determined by use of a software program.[o] A P value ≤ 0.05 was considered significant.

Results

Dogs with DIC—Blood samples were collected from 29 dogs suspected of having DIC. Of these, 20 fulfilled our predefined clinical and laboratory criteria for DIC and were included in the study. The 20 dogs with DIC were 2 to 12 years old (median age, 8 years) and included 5 Labrador Retrievers, 4 Golden Retrievers, 4 German Shepherd Dogs, 2 mixed-breed dogs, and 1 each of the following breeds: Portuguese Water Dog, Bichon Frise, Pomeranian, Siberian Husky, and Bulldog. Eleven dogs were male (4 sexually intact males, 7 castrated males), and 9 were spayed females. Bleeding was detected at 2 to 5 unrelated sites, including mucosal surfaces (petechiae, gingival bleeding, epistaxis, hematuria, melena, hematochezia, and hematemesis), subcutaneous tissues (bruising and hematomas), body cavities (thoracic and abdominal cavities), lungs, and CNS. Dogs bled excessively after venipuncture and from traumatic or surgical wounds. Diagnoses were neoplasia (hemangiosarcoma, lymphoma, multiple myeloma, and carcinoma; n = 14), hepatitis (4), acute hemorrhagic pancreatitis (1), and glomerulonephritis and acute aspiration pneumonia (1). Diagnoses were made on the basis of combinations of laboratory test results and clinical evaluation (physical examination, radiography, and ultrasonography; n = 9), and on cytologic or histologic examination of body tissues (11). Median platelet count was 46×10^9/L, with a range of 4 to 156×10^9/L. The APTT was prolonged in 95% (19/20; median APTT, 24 seconds; range, 14 to > 90 seconds; reference range, 10 to 17 seconds) of dogs, PT was prolonged in 35% (7/20; median PT, 16 seconds; range, 14 to > 90 seconds; reference range, 13 to 18 seconds) of dogs, and AT activity was low in 90% (18/20; median AT activity, 49%; range, 28 to 84%; reference range, 70 to 126%) of dogs. Mild to moderate erythrocyte fragmentation was observed in 75% of dogs with DIC.

Healthy dogs—The 30 clinically healthy dogs were between 10 months and 8 years old (median age, 4.4 years). There were 13 mixed-breed dogs, 4 Labrador Retrievers, 4 Australian Shepherds, 3 Greyhounds, 2 Golden Retrievers, 2 Dalmatians, 1 Border Collie, and 1 American Staffordshire Terrier. Thirteen were male (2 sexually intact males, 11 castrated males), and 17 were female (10 sexually intact females, 7 spayed females).

Immunoturbidometric D-dimer assay—Intra-assay coefficient of variation ranged from 2.7 to 14.7%. Inter-assay coefficient of variation ranged from 2.7 to 14.7% for canine samples and from 6.3 to 20.8% for human control sera. D-dimer concentrations in 30 healthy dogs were not normally distributed. After removal of 1 apparent outlier (D-dimer concentration, 0.52 µg/ml), a reference range of

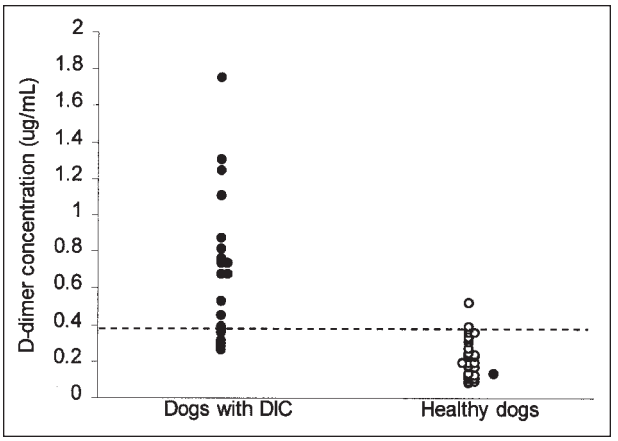

Figure 2—latex agglutination results (● = positive result; O = negative result) and immunoturbidometric D-dimer concentrations in dogs with disseminated intravascular coagulopathy (DIC n = 20) and healthy dogs (30). Dotted line indicates upper limit of reference range for the immunoturbidometric D-dimer assay (0.39 µg/ml).

Table 1—Point estimates and 95% confidence intervals (CI) of sensitivity and specificity of D-dimer and serum and plasma fibrin-fibrinogen degradation products (FDP) concentrations determined by various assays in dogs with disseminated intravascular coagulation (DIC; n = 20) and healthy dogs (30)

	Sensitivity (%)		Specificity (%)	
Assay	**Estimate**	**CI**	**Estimate**	**CI**
TW* serum FDP	85	62–97	100	88–100
SP* serum FDP	95	75–100	100	88–100
Plasma FDP	90	68–99	90	73–98
Latex agglutination D-dimer	100	83–100	97	83–100
IT D-dimer > 0.30 µg/ml	85	62–97	77	58–90
IT D-dimer > 0.39 µg/ml	65	41–85	97	83–100

*Serum FDP concentrations were measured by use of 2 latex agglutination kits (TW and SP). D-Dimer concentrations were measured by use of a latex agglutination kit[c] and an immunoturbidometric (IT) assay[i]; cutoff concentrations for the IT assay were selected on the basis of the receiver-operator characteristic curve for D-dimer concentrations (> 0.30 µg/ml) or the upper limit of the reference range determined from D-dimer concentrations of 30 healthy dogs (> 0.39 µg/ml).
IT = Immunoturbidometric.

0.08 to 0.39 µg/ml was established. D-dimer concentrations were significantly higher ($P < 0.001$) in dogs with DIC (median concentration, 0.67 µg/ml; range, 0.26 to 1.75 µg/ml; n = 20) than in healthy dogs (median concentration, 0.19 µg/ml, range, 0.08 to 0.52 µg/ml; n = 30), although ranges overlapped (**Fig 2**).

Sensitivity and specificity of individual tests—The latex-agglutination D-dimer assay was 100% sensitive in 20 dogs with DIC and 97% specific in 30 healthy dogs (**Table 1**). Serum and plasma FDP concentration assays had lower sensitivities, but these differences were not significant (FDP point estimates lay within confidence intervals for the latex-agglutination D-dimer assay). One healthy dog had positive agglutination reactions for latex-agglutination D-dimer and plasma FDP assays (plasma FDP concentration, 5 to 20 µg/ml; immunoturbidometric D-dimer concentration, 0.13 µg/ml), but a negative reaction for the serum FDP assay. The 2 D-dimer cutoff concentrations used to estimate sensitivity and specificity of the immunoturbidometric D-dimer assay were 0.30 µg/ml (derived from the receiver-operator characteristic curve [**Fig 3**]) and 0.39 µg/ml (the upper limit of the reference range). At the 0.30 µg/ml cutoff, the immunoturbidometric D-dimer assay was as sensitive as the latex-agglutination D-dimer and plasma and serum FDP assays, but less specific (Table 1). At the 0.39 µg/ml cutoff, the immunoturbidometric D-dimer assay was significantly less sensitive, but as specific, as the latex-agglutination D-dimer and SP serum and plasma FDP assays (Table 1).

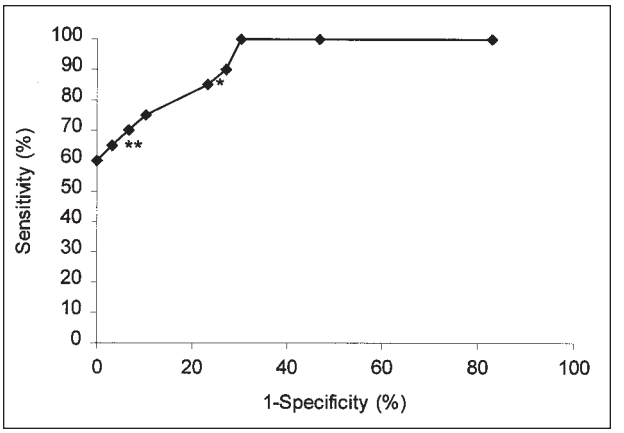

Figure 3—Receiver-operator characteristic curve for D-dimer concentrations in dogs, determined by use of an immunoturbidometric assay. * = 0.30 µg/ml D-dimer concentration cutoff. ** = 0.39 µg/ml D-dimer concentration cutoff.

Sensitivity and specificity of tests in parallel or series—When results from the latex-agglutination D-dimer and FDP assays were interpreted in parallel (ie, a positive test for DIC was equivalent to a positive result for either FDP or D-dimer assays), sensitivity was 100% (95% confidence interval, 83 to 100%), with a specificity of 97 to 100%. When results of the immunoturbidometric D-dimer and FDP assays were interpreted in parallel at the 0.30 µg/ml cutoff, sensitivity was 95% with a specificity of 80%. At the 0.39 µg/ml cutoff, sensitivity was 90 to 95%, with a specificity of 97%. When interpreted in series (ie, a positive test for DIC was equivalent to a positive result for D-dimer and FDP assays), latex-agglutination D-dimer and FDP assays had a sensitivity of 85 to 95% and a specificity of 97 to 100%. When immunoturbidometric D-dimer and FDP assays were interpreted in series at the 0.30 µg/ml cutoff, sensitivity was 75 to 85%, with a specificity of 93 to 100%. At the 0.39 µg/ml cutoff, sensitivity was 60 to 65%, with a specificity of 100%.

Concordance of FDP and D-dimer assays—Results from the latex-agglutination D-dimer and FDP assays differed in individual dogs with DIC (**Table 2**). No dogs had negative results for the latex-agglutination D-dimer assay and positive results for the serum or plasma FDP assays; kappa values, therefore, could not be determined for the latex-agglutination D-dimer assay. The latex-agglutination D-dimer and SP serum FDP assay had the highest concordance (95%). Only 1 dog had a positive result for the latex-agglutination D-dimer assay and a negative result for serum FDP with this kit. This dog also had negative results for serum FDP with the TW kit and for plasma FDP and had an

Table 2—Concordance (bold type) of D-dimer and serum and plasma FDP assay results in dogs with DIC (n = 20)

AssayS	Result	TW Serum FDP		SP serum FDP		Plasma FDP		IT D-dimer > 0.30 µg/ml		IT D-dimer > 0.39 µg/ml	
		+	–	+	–	+	–	+	–	+	–
Latex agglutination D-dimer	+	**17**	3	**19**	1	**18**	2	**17**	3	**13**	7
	–	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**
IT D-dimer > 0.30 µg/ml	+	**15**	2	**17**	0	**16**	1	NA	NA	NA	NA
	–	2	**1**	2	**1**	2	**1**	NA	NA	NA	NA
IT D-dimer > 0.39 µg/ml	+	**12**	2	**13**	0	**12**	1	NA	NA	NA	NA
	–	5	**1**	6	**1**	6	**1**	NA	NA	NA	NA

+ = Positive result. – = Negative result. NA = Not applicable.
See Table 1 for key.

immunoturbidometric D-dimer concentration of 0.28 µg/ml. Concordance between the 2 D-dimer assays was higher at the 0.30 µg/ml cutoff (85% concordance) than at the 0.39 µg/ml cutoff (65% concordance; Fig 2, Table 2). Concordance between the immunoturbidometric D-dimer assay (at either cutoff) and the FDP assays was poor (kappa values, < 0.4), with the exception of the 0.30 mg/ml cutoff and the SP serum FDP assay (90% concordance; kappa = 0.46, $P = 0.007$).

Reproducibility of latex-agglutination D-dimer assays in frozen samples—Samples from dogs with DIC were retested after storage at –25 C for 2 to 11 months. All samples had positive reactions for D-dimer on initial and repeat testing. Samples from the 30 healthy dogs were retested for D-dimer after storage at –25 C for 12 months. Of these dogs, 90% (n = 27) had negative and 10% (3) had weak-positive results on both kits after storage. Of these 3 dogs, 1 had a positive result and 2 had negative results initially.

Discussion

The antibodies in latex-agglutination and immunoturbidometric assays for D-dimer concentration used in the study reported here appear to react with D-dimer in canine plasma. Immunoturbidometric D-dimer concentrations were significantly higher in dogs with DIC (median, 0.67 µg/ml) than in healthy dogs (median, 0.19 µg/ml) and were similar to concentrations reported for 71 dogs with DIC (mean, 0.73 µg/ml) and 40 healthy dogs (mean, 0.13 µg/ml).[a] Intra-assay and inter-assay coefficients of variation of the immunoturbidometric assay were acceptable, with the exception of 1 sample (interassay coefficient of variation, 20.8%). In this sample, D-dimer concentration progressively increased with each assay (from 0.4 to 0.62 µg/ml) and may have affected result interpretation. The high variation in this sample may have been caused by sample instability because of refrigeration.

The latex-agglutination D-dimer assays evaluated in the study reported here were 100% sensitive and 97% specific for the diagnosis of DIC in our cohorts of dogs. These results were comparable to those for the serum and plasma FDP kits we evaluated. One healthy dog had a positive result on the latex-agglutination D-dimer assay; this dog also had a weak-positive reaction on the plasma FDP assay (FDP concentration, 5 to 20 µg/ml) but had negative results on both serum FDP assays. This dog was clinically normal, and we considered these to be false-positive results. The immunoturbidometric D-dimer assay did not perform as well as the latex-agglutination assay. At the 0.30 µg/ml cutoff, sensitivity was comparable (85%) to that of the latex-agglutination D-dimer and FDP assays, at the expense of specificity (77%). At the 0.39 µg/ml cutoff, the assay had significantly lower sensitivity (65%), but similar high specificity (97%), to the latex-agglutination D-dimer and SP serum and plasma FDP assays. Sensitivity of the immunoturbidometric D-dimer assay at both cutoffs in this study was similar to that reported for 71 (82%)[a] and 102 dogs with DIC (77%),[b] using a 0.25 µg/ml cutoff (upper limit of the reference range). Using the upper limit of our reference range (0.39 µg/ml cutoff), specificity in our study (97%) was not substantially different from that reported for 40 healthy dogs (100%),[a] despite our reference range being broader.

Most laboratory results from an individual patient are interpreted with respect to reference ranges that should be established by the laboratory. In our laboratory, the upper limit of the reference range for the immunoturbidometric D-dimer assay was 0.39 µg/ml. As mentioned, this cutoff had low sensitivity (65%; confidence interval, 41 to 85%). Sensitivity was improved significantly (to 90 or 95%; ie, outside the confidence limits of the point estimate when used alone), without loss of specificity, when used in parallel with serum or plasma FDP assays. At the cutoff of 0.30 µg/ml, specificity of the immunoturbidometric D-dimer assay was significantly improved when combined with FDP assays in series. Our data suggests that the immunoturbidometric D-dimer assay could be used simultaneously with FDP assays but should not replace them as a diagnostic test for DIC in dogs. Sensitivity of the FDP assays was improved, although not always significantly, when used in parallel with the D-dimer assays. These data suggest that if there is a high clinical suspicion for DIC, and results of other laboratory tests are compatible with DIC in a particular patient, but serum or plasma FDP assay results are negative, a positive result for D-dimer (by latex-agglutination or immunoturbidometry) would nevertheless support the diagnosis of DIC.

The latex-agglutination D-dimer and FDP assays yielded concordant results in most dogs with DIC. In contrast, concordance was poorer with the immunoturbidometric D-dimer assay (especially at the 0.39 µg/ml cutoff) and both latex agglutination D-dimer and FDP

assays. Between 1 to 3 dogs had positive results with the latex agglutination D-dimer assay but had negative results for the serum or plasma FDP assay. This can be attributed to adsorption of FDP during clot formation in vitro in the collection tubes for the serum FDP assay or to low sensitivity of FDP assays to crosslinked degradation products containing D-dimer epitopes.[16]

According to manufacturers of the latex agglutination kits, D-dimer is stable in samples from human patients stored at –20 C for up to 1 month after collection. Our results indicate that D-dimer can be measured in canine citrate plasma samples stored at –25 C for longer than 1 month. There were no false-negative reactions for D-dimer in dogs with DIC after storage of samples for 2 to 11 months. Also, most samples from healthy dogs had repeat negative results for D-dimer after storage for 12 months (only 2 dogs had new false-positive reactions). This suggests that canine samples can be frozen for longer than 1 month and still provide accurate D-dimer results. However, we have noted that D-dimer concentrations appear to progressively decrease in canine samples with multiple freeze-thaws. Therefore, further studies are needed to determine effects of multiple freeze-thaw cycles and duration of storage on D-dimer concentrations in canine samples.

Our data suggest that the latex agglutination D-dimer assay could replace FDP as the laboratory test for fibrinolysis in dogs suspected of having fulminant- or hemorrhagic-phase DIC. This assay was more sensitive than serum or plasma FDP assays, but was equally specific. However, further studies are required to evaluate the specificity of FDP and D-dimer assays in dogs with systemic disorders other than DIC. A preliminary study[b] suggested that the plasma FDP assay has a specificity of 84% in a similar population. In that study, the immunoturbidometric D-dimer assay had a specificity of 95%. In humans, high D-dimer concentrations are not specific for DIC; increased concentrations may be detected in conjunction with several other conditions, including deep vein thrombosis, neoplasia, and extravascular fibrinolysis associated with postoperative wound healing.[7,8,14] Therefore, until further information on clinical patients is known, D-dimer and FDP tests should be used simultaneously as supportive tests for the diagnosis of DIC in dogs and for evidence of plasmin's degradation of fibrin or fibrinogen.

[a]Caldin M, Furlanello T, Berto D, et al. Preliminary investigations of D-dimer concentrations in normal dogs and dogs with disseminated intravascular coagulation (DIC; abstr). *J Vet Intern Med* 1997;11:130.
[b]Caldin M, Furlanello T, Lubas G. Sensitivity and specificity of citrated plasma FDPs and D-dimer in the diagnosis of disseminated intravascular coagulation (DIC) in dogs (abstr). *J Vet Intern Med* 1998;12:236.
[c]D-dimer assay, Pacific Hemostasis, Huntersville, NC.
[d]Accuclot D-dimer, Sigma Diagnostics, St Louis, Mo.
[e]Tina-quant A D-dimer, Boehringer Mannheim Corp, Indianapolis, Ind.
[f]Hitachi 911, Boehringer Mannheim Corp, Indianapolis, Ind.
[g]Thrombo-Wellcotest, Murex Diagnostics, Norcross, Ga.
[h]Spli-Prest, Diagnostica Stago, American Bioproducts Co, Parsippany, NJ.
[i]FDP plasma, Diagnostica Stago, American Bioproducts Co, Parsippany, NJ.
[j]Coulter S+IV Hematology Analyzer, Coulter Electronics, Hialeah, Fla.
[k]Thromboscreen, Pacific Hemostasis, Huntersville, NC.
[l]Dade Actin FS Reagent, Baxter Diagnostics, Edison, NJ.
[m]Coatest Antithrombin, diaPharma, West Chester, Ohio.
[n]Statistix for Windows, Analytical Software, Tallahassee, Fla, 1996.
[o]Epi Info Version 6.04b, Center for Disease Control and Prevention, Atlanta, Ga, 1997.

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