Clinical Use of D-Dimer Measurement for the Diagnosis of Disseminated Intravascular Coagulation in Dogs

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ABSTRACT. We measured the plasma D-dimer (DD) concentration in 205 dogs. Simultaneously, fibrinogen/fibrin degradation products (FDPs) concentration, platelet (PLT) count, prothrombin time (PT), activated partial thromboplastin time (APTT), and plasma concentrations of fibrinogen (Fib) were measured in the same dogs. From these results, we were able to divide the animals into four groups: control (healthy dogs, n=18), pre-disseminated intravascular coagulation (preDIC) (n=20), disseminated intravascular coagulation (DIC) (n=21), and other (n=146). Significant differences in plasma DD concentration were found among the four groups: control, 0.45 ± 0.46 mg/ml (reference range, 0–1.37); preDIC, 5.0 ± 4.8 mg/ml; DIC, 16.3 ± 12.2 mg/ml; and other, 1.5 ± 2.7 mg/ml. A weak positive correlation (r=0.62) was found between FDPs and DD concentrations in the DIC group. As a DIC diagnostic test, the PLT/APTT/FDPs/DD combination had the highest accuracy of 100%, with a sensitivity of 73% and a specificity of 97%. We propose the use of FDPs and DD concentrations as part of the DIC diagnostic test panel, with DD and FDPs to provide accurate diagnosis.

KEY WORDS: canine, D-dimer (DD), disseminated intravascular coagulation (DIC), fibrinogen/fibrin degradation products (FDPs)

Disseminated intravascular coagulation (DIC) is a secondary disease that is always initiated by an underlying and primary pathologic process [2, 5, 10, 17]. Moreover, the status of a patient with DIC changes over time and therefore the results of a laboratory coagulation test may fluctuate rapidly and repeatedly. Most veterinarians have no clear concept of the rational laboratory approach to the diagnosis of DIC. They order a multitude of tests, most of which are not specific, and follow up the course of this condition by repeating the same tests. In the treatment of human patients with DIC, the combination of fibrinogen/fibrin degradation products (FDPs) and D-dimer (DD) has been reported to offer the best molecular markers for understanding the process of systemic activation of the procoagulant and secondary fibrinolytic systems in DIC [6, 12–14].

DD is pure cross-linked fibrin degradation products. Therefore, an increase in DD concentration is specific for the combined presence of coagulation and fibrinolysis (physiologic or pathologic). In contrast, an increase in the concentration of FDPs is not specific for active coagulation because FDPs include both fibrin and fibrinogen degradation products. However, when the concentration of FDPs is increased, active fibrinolysis is demonstrated [1, 2, 5, 7]. Turbidimetric immunooassay (TIA) and latex agglutination, which uses anti-human DD mouse monoclonal antibody, have been reported for the measurement of DD concentration in dogs [3, 9, 13–16]. In these studies, DD was found to be a useful marker for the diagnosis of DIC. Assays of latex agglutination DD and serum and plasma FDPs had similar sensitivity and specificity; the immunoturbidimetric assay had lower specificity (77%) at the 0.30 mg/ml cut-off and lower sensitivity (65%) at the 0.39 mg/ml cut-off [16]. It was shown that evaluation was different depending on the method for measuring DD concentration. Moreover, DD concentration differed depending on the measurement kit used.

There have been many reports of the measurement of DD concentration in the dog in different countries [3, 9, 13–16]. However, few such studies have been conducted in Japan. Therefore, the aim of this study was to examine the utility of DD concentration measurement in dogs with DIC with a reagent that could be used in Japan.

MATERIALS AND METHODS

**Animals:** The study subjects included 205 dogs presented to the Rakuno Gakuen University Veterinary Teaching Hospital. These 205 dogs included 34 dog species, with 114 males (22 castrated) and 91 females (26 spayed).

**Blood:** Blood was collected from the jugular, cephalic, or saphenous vein. Blood samples were obtained in following order: 1) 1 ml in a tube containing ethylenediaminetetraacetic acid (EDTA); 2) 1 ml in a tube containing 3.8% sodium acid citrate; and 3) 1 ml in a vacuum tube containing thrombin, aprotinin, and snake venom. Centrifugation (2,000 rpm, 15–25°C, 10 min) of samples (except those in tubes containing EDTA) was completed within 30 min of sample collection. The measurements of platelet (PLT) count, prothrombin time (PT), activated partial thromboplastin time (APTT), and plasma concentrations of fibrinogen (Fib), FDPs, and DD were carried out within 2 hr of sample prep-
aration.

*Measurements:* PLT count was measured with an automatic blood cell counter (MICROS abc LC-152 Horiba, Kyoto, Japan). PT, APTT, and plasma Fib concentration were measured with a semi-automatic mechanical clot detection system (DRIHEMATO® system CG 02V, A&T, Kanagawa, Japan). FDPs and DD concentrations were measured with BECKMAN-COULTER SYNCHRON Clinical System CX® 7A (Beckman-Coulter, Brea CA, U.S.A.). Anti-human Fib rabbit polyclonal antibody was used for the measurement of serum FDPs concentration, and anti-human DD mouse monoclonal antibody was used for the measurement of plasma DD concentration. Both were measured using latex agglutination assay.

*Dilution linearity of the DD measurement reagent:* Three plasma samples from dogs with different ranges of plasma DD concentration (18.72, 9.42, and 3.05 {g/mL}) were diluted with the standard dilution solution and the concentration of each diluted sample was determined in triplicate.

*Diagnostic criteria for DIC or preDIC:* DIC was diagnosed by four or more abnormal findings among the following: low PLT count (<200 x 10^3/μl), prolonged PT (>8.0 sec) or APTT (>18.0 sec), low Fib (<150 mg/dl) or high FDPs (≥10 μg/mL) concentration, and the presence of schistocytes. PreDIC was diagnosed by three of the above [4, 5, 7].

*Correlation of clotting factors in the control, preDIC, DIC, and other group:* We divided the 205 dogs, presented to the Rakuno Gakuen University Veterinary Teaching Hospital, into a control group (n=18; 9 males and 9 females; age, 1–8 years; body weight, 3–30 kg), preDIC group (n=20; 9 males and 11 females; age, 1–13 years; body weight, 3–30 kg), DIC group (n=21; 14 males and 7 females; age, 5–13 years; body weight, 3–30 kg), and other group (n=146; 75 males and 71 females; age, 1–15 years; body weight, 3–30 kg). The control group included clinically healthy dogs with normal clotting factor measurements, and the other group included all clinically unhealthy dogs that did not fit into the control, DIC, or preDIC group. Clotting factors were compared between the four groups. Using the results of measurement with six items (i.e., PLT count, PT, APTT, and concentrations of Fib, FDPs, and DD), 57 combination patterns (the combination of two items, 15 patterns; combination of three items, 20 patterns; combination of four items, 15 patterns; combination of five items, 6 patterns; combination of six items, 1 pattern) were obtained. The control and DIC groups were compared and the combination patterns that were significantly different between the groups were evaluated [18].

*Statistics:* The normal distribution of the data was evaluated by the χ² test, and differences between groups were analyzed by the Mann-Whitney test. A P value <0.05 was considered significant. We used a linear discriminant function (variable selection) to ascertain the 57 combination patterns with the largest difference between the control and DIC groups in discriminant analyses. Receiver operating characteristic (ROC) analysis was carried out in order to consider the reliability of DD and FDPs measurement. Reliability is expressed as area under the ROC curve (AUC). An evaluation value is set to a maximum of 1 and at least 0.5. Reliability is high and the value exceeding 0.8 is considered excellent [8, 11].

**RESULTS**

*Dilution linearity of the DD measurement reagent:* As can be seen in Fig. 1, the analysis of three plasma samples to check the linearity under assay dilution resulted in linear regression equations with correlation coefficients higher than 0.99.

*Differences in the coagulation tests in control, preDIC, DIC, and other group:* Table 1 shows that the results of coagulation tests were significantly different between groups. Figure 2 shows that FDPs and DD concentrations were significantly different between groups. In the control group, the serum concentration of FDPs was 0.46 ± 0.72 μg/ml (reference range, 0–1.90 μg/ml), and the plasma DD concentration was 0.45 ± 0.46 μg/ml (reference range, 0–1.37 μg/ml). The concentrations of FDPs in the preDIC, DIC, and other groups were 5.0 ± 4.8, 16.3 ± 12.2, and 1.5 ± 2.7 μg/ml, respectively. FDPs and DD concentrations were significantly higher in the preDIC and DIC groups than in the control and other groups, and the DIC group showed significantly higher mean FDPs and DD concentrations than the preDIC group (Fig. 2). PLT count, PT, and APTT were significantly different in the preDIC and DIC groups compared with the control and other groups (Table 1).

![Fig. 1. Dilution linearity of DD concentration. Three plasma samples from the dogs with different ranges of DD concentration were diluted with the standard dilution solution and the concentration of each diluted sample was determined in triplicate. ▲, 18.72 μg/mL, y=4.745x+0.031, r=0.9989. ■, 9.42 μg/mL, y=2.394x+0.029, r=0.9954. ●, 3.05 μg/mL, y=0.764x+0.045, r=0.9945.](image-url)
Correlation between FDPs and DD concentrations: The correlation between FDPs and DD concentrations in the 205 samples (including healthy dogs and those with various diseases) was high (y=0.99x+2.25, r=0.79, n=205) (Fig. 3). In contrast, when the correlation between FDPs and DD concentrations in the DIC group was investigated, only a weak positive correlation was found (y=0.47x+11.9, r=0.62, n=21) (Fig. 4).

ROC analysis: The ROC curves of FDPs and DD concentrations (control, n=18; DIC, n=21) are shown in Fig. 5. The AUC of FDPs (0.991) indicated that the value was better than the AUC of DD (0.938). The measurement of FDPs and DD concentrations was shown to be superior in terms of the degree of contribution to DIC diagnosis.

Comparison of measurement of the coagulation system in the control and DIC groups: When we examined the order for the best combination of measurement with 6 items (i.e., PLT count, PT, APTT, and plasma concentrations of Fib, FDPs, and DD), we found that the combination of PLT/APTT/ FDPs/DD was superior to the other 56 combinations (Table 2). This combination had the highest diagnostic accuracy of 100%, with a sensitivity of 73% and a specificity of 97%.

DISCUSSION

Latex agglutination and immunoturbidometric assays have been reported to be suitable methods for measuring DD concentration in dogs [3, 9, 13–16]. These assays have been developed for human use and therefore, in this study, DD concentration in dogs has been determined using a reagent for human measurement that could be used in Japan. It appears that this DD measurement reagent made and used in Japan is different from the reagents used in a number of other countries.

Excellent linearity was demonstrated with all the dilutions tested, which means that the accuracy of the automated method is acceptable.
The average DD concentration ($\pm 2SD$) of the control group showed a lower limit of 0 $\mu g/ml$ and an upper limit of 1.37 $\mu g/ml$ (mean, 0.45 $\mu g/ml$). The reference concentration for a healthy dog reported here was higher than that described previously (e.g., mean, 0.13, 0.19, or <0.25 $\mu g/ml$) [3, 14–16]. The average DD concentration of the DIC group was 16.3 $\pm 12.2$ $\mu g/ml$ (mean $\pm SD$). This concentration is higher than that reported previously (e.g., mean, >0.25 to 0.67 $\mu g/ml$) [13–16]. The reason for this difference was thought to be due to the laboratory procedures, for example, differences in the assay methods, detection of antibodies, and calibrators used. In addition, different studies have used different cut-off values for determining the sensitivity and specificity of a test. Therefore, results from different systems are not directly comparable in dogs. However, the data strongly suggest that DD concentration is high in DIC in dogs. Therefore, the quantitative measurement of DD concentration is useful as a diagnostic indicator of DIC [9, 13–16].

The plasma DD concentration was higher in the DIC group than in the preDIC group. Moreover, there was a significant difference in DD concentration between all four groups. Similar results for serum FDPs concentration were found.

When the correlation between serum FDPs and plasma DD concentrations was investigated in all samples (205 dogs), we found a strong positive association. In contrast, the correlation between FDPs and DD concentrations in the DIC group was weak. In several dogs in the DIC group, we found a marked increase in FDP concentration, but no change in DD concentration (data are not shown). Because FDPs and DD concentrations in the DIC group do not necessarily show a high correlation, they should be measured independently. DIC is a complication of various diseases. Moreover, it constitutes a dynamic phenomenon during which a patient’s status and laboratory coagulation test results may fluctuate markedly and repeatedly. Therefore, it is important to measure both FDPs and DD concentration changes.

Additionally, in the ROC analysis, the superiority of both serum FDPs and plasma DD concentrations for DIC diagnosis was found with the AUC values (0.991 and 0.938, respectively). To determine the diagnostic importance of plasma DD concentration for differentiating DIC and non-DIC, a cut-off value (1.37 $\mu g/ml$) was established by analyzing ROC curves in the present study. As a result, the sensitivity and specificity was acceptable for a clinical DIC test.

Correlation analysis with a combination of each coagulation measurement item in the control and DIC groups showed that the most significant difference was obtained with the combination of PLT/APTT/FDPs/DD (accuracy, 100%; sensitivity, 73%; specificity, 97%) (Table 2). Thus, a combination of measurement items including DD concentration was shown to be effective for the diagnosis of DIC.

From the above results, it appears that latex agglutination assay with anti-human DD mouse monoclonal antibody as used in this study was a suitable method for the measurement of DD concentration in the dog. We suggest that a plasma DD concentration of 1.37 $\mu g/ml$ or more would be a useful diagnostic criterion for DIC. Measurement of DD concentration by latex agglutination appears to be a sensitive and specific ancillary test for DIC in dogs. The specificity for DD concentration in dogs with systemic diseases other than DIC has not yet been determined; therefore, assays of FDPs and DD should be performed concurrently as supportive tests for the diagnosis of DIC in dogs.

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PLASMA D-DIMER IN DOGS WITH DIC

Fig. 5. Relative operating characteristic (ROC) curves of the plasma DD and serum FDPs concentrations. A, plasma DD concentration; B, serum FDPs concentration. AUC expresses the area under the ROC curve.

Table 2. Comparison of utility of combination of coagulation tests in DIC

<table>
<thead>
<tr>
<th>Rank</th>
<th>Combination</th>
<th>Wilks’s Lambda</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PLT/APTT/FDPs/DD</td>
<td>0.07805</td>
<td>73</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>PT/APTT/FDPs/DD</td>
<td>0.08459</td>
<td>71</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>PLT/PT/FDPs/DD</td>
<td>0.08549</td>
<td>71</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>PLT/FDPs/DD</td>
<td>0.10227</td>
<td>69</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>PLT/Fib/FDPs/DD</td>
<td>0.10422</td>
<td>71</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>6</td>
<td>PT/Fib/FDPs/DD</td>
<td>0.10422</td>
<td>68</td>
<td>100</td>
<td>93</td>
</tr>
<tr>
<td>7</td>
<td>APTT/Fib/FDPs/DD</td>
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<td>71</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>8</td>
<td>APTT/FDPs/DD</td>
<td>0.11003</td>
<td>69</td>
<td>100</td>
<td>93</td>
</tr>
<tr>
<td>9</td>
<td>PLT/PT/APTT/FDPs</td>
<td>0.11381</td>
<td>73</td>
<td>95</td>
<td>98</td>
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<tr>
<td>10</td>
<td>PT/FDPs/DD</td>
<td>0.11781</td>
<td>66</td>
<td>100</td>
<td>93</td>
</tr>
</tbody>
</table>

PLT, platelet; PT, prothrombin time; APTT, activated partial thromboplastin time; FDPs, fibrinogen/fibrin degradation products; DD, D-dimer; Fib, fibrinogen.

The control and DIC groups were compared and the 57 combination patterns that were significantly different between the control and DIC groups were evaluated. We used a linear discriminant function (variable selection) to ascertain the combination items with the largest difference between the groups in discriminant analyses. We listed the 10 best combinations from the 57 possible combinations.

REFERENCES


