Comparison of prothrombin time, activated partial thromboplastin time, and fibrinogen concentration in blood samples collected via an intravenous catheter versus direct venipuncture in dogs

Vera A. Maeckelbergh, DVM, and Mark J. Acierno, MBA, DVM

Objective—To compare prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen concentration in canine blood samples collected via an indwelling IV catheter and direct venipuncture.

Animals—35 dogs admitted to an intensive care unit that required placement of an IV catheter for treatment.

Procedures—Blood samples were collected via IV catheter and direct venipuncture at the time of catheter placement and 24 hours after catheter placement. Prothrombin time, APTT, and fibrinogen concentration were measured.

Results—5 dogs were excluded from the study; results were obtained for the remaining 30 dogs. Agreement (bias) for PT was −0.327 seconds (limits of agreement, −1.350 to 0.696 seconds) and 0.003 seconds (limits of agreement, −1.120 to 1.127 seconds) for the 0- and 24-hour time points, respectively. Agreement for APTT was −0.423 seconds (limits of agreement, −3.123 to 2.276 seconds) and 0.677 seconds (limits of agreement, −3.854 to 5.207 seconds) for the 0- and 24-hour time points, respectively. Agreement for fibrinogen concentration was −2.333 mg/dL (limits of agreement, −80.639 to 75.973 mg/dL) and −1.767 mg/dL (limits of agreement, −50.056 to 46.523 mg/dL) for the 0- and 24-hour time points, respectively.

Conclusions and Clinical Relevance—Agreement between the 2 techniques for sample collection was clinically acceptable for PT, APTT, and fibrinogen concentration at time 0 and 24 hours. It is often difficult or undesirable to perform multiple direct venipunctures in critically ill patients. Use of samples collected via an IV catheter to monitor PT and APTT can eliminate additional venous trauma and patient discomfort and reduce the volume of blood collected from these compromised patients. (Am J Vet Res 2008;69:868–873)

Abbreviations

<table>
<thead>
<tr>
<th>APTT</th>
<th>Activated partial thromboplastin time</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>Prothrombin time</td>
</tr>
</tbody>
</table>

To our knowledge, there has been no study in veterinary medicine that examined clotting times for samples obtained via IV catheter to determine whether they are in agreement with those for samples obtained via direct venipuncture. Therefore, the objective of the study reported here was to determine whether the APTT, PT, and fibrinogen concentration of blood samples obtained via an IV catheter were in agreement with results for samples collected by direct venipuncture.

Materials and Methods

Dogs—Thirty-five client-owned dogs examined at our university veterinary teaching hospital between September 2006 and May 2007 were included in the study. Dogs were selected for inclusion when treatment for their condition required that an IV catheter be placed and when they were expected to remain in the intensive care unit for at least 24 hours. No attempt was made to screen dogs on the basis of age, sex, type of disease, or severity of illness. Patients from which blood samples could not be obtained...
at the time of catheter placement and at 24 hours were excluded from the study. The School of Veterinary Medicine Clinical Study Protocol Review Committee approved the study. Consent was obtained from the owner of each participating dog.

Procedures—Initial blood samples were collected at the time that a polyvinylchloride central venous catheter was placed (time 0). All IV catheters were located in a jugular vein or a lateral saphenous vein. A 2.7-mL blood sample was aspirated through the IV catheter. Concurrently, a 2.7-mL blood sample was collected by direct venipuncture of the contralateral jugular or lateral saphenous vein or a cephalic or medial saphenous vein. Collection of blood by direct venipuncture was performed with a 20- or 22-gauge needle and 3-mL syringe. Once collected, blood samples were immediately placed into blood collection tubes containing 3.2% sodium citrate. For a small number of dogs, instead of the use of the technique in which 2.7 mL of blood was collected, smaller samples of 0.9 mL were collected via direct venipuncture at 0 or 24 hours (or both times) by use of a 22-gauge needle and a 1-mL syringe containing 0.1 mL of 3.2% sodium citrate; these samples were then transferred to collection tubes that did not contain any anticoagulant.

Twenty-four hours after collection of the initial blood samples, the process was repeated but with the following modification: the blood sample collected via the IV catheter at 24 hours was obtained by use of a standard 3-syringe technique. For this technique, 0.5 mL of heparinized saline (0.9% NaCl) solution was injected into the IV catheter. Then, 3 mL of blood was aspirated from the catheter and set aside. The 2.7-mL blood sample was then collected via the IV catheter and placed into a tube containing sodium citrate. The 3 mL of blood was then injected back into the catheter, which was followed by a flush with 3 mL of heparinized saline solution.

All samples were immediately transferred to our veterinary clinical pathology laboratory for analysis. At the laboratory, all samples were centrifuged. For samples that could not be analyzed immediately, the plasma was harvested and frozen at −30°C until analyzed. All frozen samples were thawed and analyzed within 72 hours. Samples were processed in paired fashion such that concurrent samples (the sample collected via the IV catheter and the sample collected simultaneously via direct venipuncture) were analyzed at the same time by the same laboratory technician. All samples were analyzed with the same coagulation analyzer. The analyzer was capable of measuring clotting times by mechanical and optical methods. In the study reported here, mechanical principles (the ball method) were used to determine values for PT and APTT and the fibrinogen concentration. Reference ranges for dogs were those that had been established previously by the veterinary clinical pathology laboratory by use of the ball method on blood samples obtained from clinically normal dogs.

Statistical analysis—Measurements of PT, APTT, and fibrinogen concentration were analyzed for agreement by use of the Bland-Altman method. Bias was defined as the mean difference between the 2 methods. Limits of agreement were defined as the bias ± (1.96 × SD). Data were analyzed by use of a commercially available statistical software program.

Results

Of the 35 dogs enrolled in the study, 5 were excluded on the basis of the aforementioned criteria. The remaining 30 dogs ranged from 6 months to 13 years of age (median, 6 years). Breeds represented were Labrador Retrievers (n = 5), Golden Retrievers (4), Boxers (4), Miniature Schnauzers (2), Yorkshire Terriers (2), mixed-breed dogs (2), Belgian Malinois (1), Blue Heeler (1), Borzoï (1), Catahoula Leopard Dog (1), Dachshund (1), Dalmatian (1), Jack Russell Terrier (1), Maltese (1), Papillon (1), Shetland Sheepdog (1), and Shih Tzu (1). There were 13 males and 17 females. The dogs were admitted to the intensive care unit for various underlying conditions, including neoplasia (n = 11), gastrointestinal or pancreatic disease (5), hepatic disease (2), snake or insect bite (2), seizures (2), congestive heart failure (1), congestive heart failure and chronic renal failure (1), diabetic ketoacidosis and pancreatitis (1), immune-mediated polychondritis (1), protein-losing enteropathy (1), pulmonary contusion (1), weakness or collapse (1), and anticoagulant rodenticide intoxication (1).

Measurement of PT for samples collected via direct venipuncture at the time of IV catheter placement (time 0) ranged from 7.8 to 47 seconds (mean, 11.5 seconds). The reference range for PT by use of the laboratory analyzer was 7.5 to 10 seconds. Of the 30 samples collected via direct venipuncture at time 0, 15 (50%) had a mild increase in PT, 1 (3%) had a severe increase in PT, and 1 (3%) had a mild increase in PT (value was between 10 and 15 seconds), and 1 (3%) had a severe increase in PT. The PT measurements for samples collected via the IV catheter at time 0 ranged from 7.9 to 45.6 seconds (mean, 11.0 seconds). Of those 30 samples, 10 (33%) had a mild increase in PT (value was between 10 and 15 seconds) and 1 (3%) had a severe increase in PT.

The PT measurements for samples collected via direct venipuncture at 24 hours ranged from 7.6 to 12.2 seconds (mean, 9.5 seconds). Eight of those 30 (27%) samples had a mild increase in PT. The PT measurements for samples collected via the IV catheter at 24 hours ranged from 8.2 to 12.2 seconds (mean, 9.6 seconds). Seven of those 30 (23%) samples had a mild increase in PT. No samples collected by either method had a severe increase in PT.

Bland-Altman analysis of PT at time 0 revealed a bias between the 2 methods of −0.327 seconds, with a limit of agreement of −1.350 to 0.696 seconds (Figure 1). Analysis of PT at 24 hours revealed a bias between the 2 methods of 0.003 seconds, with a limit of agreement of −1.120 to 1.127 seconds (Figure 2).

The APTT measurements for samples collected via direct venipuncture at time 0 ranged from 10.9 to 120 seconds (mean, 20.6 seconds). The reference range for APTT by use of the laboratory analyzer was 11.0 to 14.0 seconds. Of the 30 samples collected via direct venipuncture at time 0, 24 (80%) had a mild increase in APTT (value was between 14 and 25 seconds), and 2 (7%) had a severe increase in APTT. The APTT measurements for samples collected via the IV catheter at time 0 ranged from 11.2 to 120 seconds (mean, 20.1 seconds).
seconds). Of those 30 samples, 20 (67%) had a mild increase in APTT and 3 (10%) had a severe increase in APTT.

The APTT measurements for samples collected via direct venipuncture at 24 hours ranged from 11.3 to 24.4 seconds (mean, 16.5 seconds). Of those 30 samples, 21 (70%) had a mild increase in APTT and 2 (7%) had a severe increase in APTT. The APTT measurements for samples collected via the IV catheter at 24 hours ranged from 10.6 to 31.1 seconds (mean, 17.2 seconds). Of those 30 samples, 21 (70%) had a mild increase in APTT and 2 (7%) had a severe increase in APTT.

Bland-Altman analysis of APTT at time 0 revealed a bias between the 2 methods of –0.423 seconds, with a limit of agreement of –3.123 to 2.276 seconds. Analysis of APTT at 24 hours revealed a bias between the 2 methods of 0.677 seconds, with a limit of agreement of –3.854 to 5.207 seconds, with a limit of agreement of –0.327 to 0.120 seconds. Of those 30 samples, 20 (67%) had a mild increase in APTT and 2 (7%) had a severe increase in APTT.

Fibrinogen concentration for samples collected via direct venipuncture at time 0 ranged from 72 to 900 mg/dL (mean, 340 mg/dL). The reference range for fibrinogen concentration by use of the laboratory analyzer was 150 to 265 mg/dL. Of the 30 samples collected via direct venipuncture at time 0, 13 (43%) had a mild increase in fibrinogen concentration (concentration was between 265 and 600 mg/dL) and 4 (13%) had a severe increase. Fibrinogen concentration for samples collected via the IV catheter at time 0 ranged from 93 to 818 mg/dL (mean, 338 mg/dL). Of those 30 samples, 12 (40%) had a mild increase in fibrinogen concentration and 4 (13%) had a severe increase in fibrinogen concentration.

Fibrinogen concentration for samples collected via direct venipuncture at 24 hours ranged from 139 to 850 mg/dL (mean, 350 mg/dL). Of those 30 samples, 16 (53%) had a mild increase in fibrinogen concentration and 2 (7%) had a severe increase in fibrinogen concentration. Fibrinogen concentration for samples collected via the IV catheter at 24 hours ranged from 147 to 866 mg/dL (mean, 348 mg/dL). Of those 30 samples, 14 (47%) had a mild increase in fibrinogen concentration.
Numerous disease conditions encountered in veterinary medicine require close monitoring of coagulation variables in affected animals. Animals with many of these conditions, such as rodenticide toxicosis, hepatic disease, immune-mediated hemolytic anemia, thrombocytopения, sepsis, and disseminated intravascular coagulation, are commonly treated in an intensive care unit where around-the-clock medical care is available. Because of the critical condition of these patients, placement of indwelling IV catheters to facilitate serial monitoring of many blood variables, such as electrolytes or glucose, is typically performed. Reliable venous access in these patients is often problematic because of the small size of the patient or the vein, underlying disease causing vessel fragility, and sequelae of multiple venipunctures. The ability to collect representative samples from an IV catheter for serial monitoring of coagulation variables would eliminate additional venous trauma, patient discomfort, and potential excessive blood loss from multiple venipuncture attempts.

The argument against the use of IV catheters for collection of samples for coagulation analysis is based on 2 principles. First, the catheter can act as a foreign irritant to the blood vessel in which it is placed. Puncture of the vein and advancement of the catheter, regardless of biocompatibility, can be responsible for the release of tissue factor and can activate the clotting cascade. Theoretically, this premature activation of blood clotting could result in falsely low clotting times for blood collected at the time of placement of an IV catheter. Additionally, the placement of an IV catheter is often more technically demanding than is simple venipuncture; this can result in a longer period of venous congestion before sample acquisition, which can also activate the clotting cascade and cause a falsely low measurement. Ideally, venous congestion should be limited prior to use of any method of sample collection to enable the accurate PT and APTT measurements.

Numerous studies have been performed in human medicine to determine the effect of various methods of sample acquisition on blood clotting variables. For example, it is often recommended that the sample used for coagulation analysis be the second or third tube of blood collected so that contamination of the sample with tissue thromboplastin possibly resulting from the venipuncture is avoided. Involving the use of clinically normal adults and adults receiving anticoagulant treatments have revealed that collection of blood into a discard tube prior to collection of the sample for coagulation analysis had no effect on measured PT and APTT values and is an unnecessary source of iatrogenic blood loss. This is especially pertinent in veterinary patients in which total blood volume is often substantially less than that of their human counterparts. In the study reported here, no attempt was made to specifically aspirate and discard a specific volume of blood prior to collection of the sample for coagulation analysis; however, the default technique that was used for collection of samples from IV catheters at 24 hours involved removal of a 3-mL volume prior to collection of the sample used for analysis.

All samples collected via direct venipuncture in this study were collected by use of 20- or 22-gauge needles and a 1- or 3-mL syringe. A 21-gauge needle is currently recommended for collection of samples for coagulation analysis in humans. Studies have been performed in human medicine to evaluate the effect of various methods of direct venipuncture, including the influence of differences in size of needle bore and col-

Figure 5—Bland-Altman plot depicting agreement of fibrinogen concentrations in samples collected via 2 methods at time 0. Bias is −1.767 mg/dL, with limits of agreement of −80.639 to 75.973 mg/dL. See Figure 1 for remainder of key.

Figure 6—Bland-Altman plot depicting agreement of fibrinogen concentrations in samples collected via 2 methods at 24 hours after placement of an IV catheter. Bias is −1.767 mg/dL, with limits of agreement of −50.056 to 46.523 mg/dL. See Figure 1 for remainder of key.

Discussion

Numerous disease conditions encountered in veterinary medicine require close monitoring of coagulation variables in affected animals. Animals with many of these conditions, such as rodenticide toxicosis, hepatic disease, immune-mediated hemolytic anemia, thrombocytopения, sepsis, and disseminated intravascular coagulation, are commonly treated in an intensive care unit where around-the-clock medical care is available. Because of the critical condition of these patients, placement of indwelling IV catheters to facilitate serial monitoring of many blood variables, such as electrolytes or glucose, is typically performed. Reliable venous access in these patients is often problematic because of the small size of the patient or the vein, underlying disease causing vessel fragility, and sequelae of multiple venipunctures. The ability to collect representative samples from an IV catheter for serial monitoring of coagulation variables would eliminate additional venous trauma, patient discomfort, and potential excessive blood loss from multiple venipuncture attempts.

The argument against the use of IV catheters for collection of samples for coagulation analysis is based on 2 principles. First, the catheter can act as a foreign irritant to the blood vessel in which it is placed. Puncture of the vein and advancement of the catheter, regardless of biocompatibility, can be responsible for the release of tissue factor and can activate the clotting cascade. Theoretically, this premature activation of blood clotting could result in falsely low clotting times for blood collected at the time of placement of an IV catheter. Additionally, the placement of an IV catheter is often more technically demanding than is simple venipuncture; this can result in a longer period of venous congestion before sample acquisition, which can also activate the clotting cascade and cause a falsely low measurement. Ideally, venous congestion should be limited prior to use of any method of sample collection to enable accurate PT and APTT measurements.

Numerous studies have been performed in human medicine to determine the effect of various methods of sample acquisition on blood clotting variables. For example, it is often recommended that the sample used for coagulation analysis be the second or third tube of blood collected so that contamination of the sample with tissue thromboplastin possibly resulting from the venipuncture is avoided. Involving the use of clinically normal adults and adults receiving anticoagulant treatments have revealed that collection of blood into a discard tube prior to collection of the sample for coagulation analysis had no effect on measured PT and APTT values and is an unnecessary source of iatrogenic blood loss. This is especially pertinent in veterinary patients in which total blood volume is often substantially less than that of their human counterparts. In the study reported here, no attempt was made to specifically aspirate and discard a specific volume of blood prior to collection of the sample for coagulation analysis; however, the default technique that was used for collection of samples from IV catheters at 24 hours involved removal of a 3-mL volume prior to collection of the sample used for analysis.

All samples collected via direct venipuncture in this study were collected by use of 20- or 22-gauge needles and a 1- or 3-mL syringe. A 21-gauge needle is currently recommended for collection of samples for coagulation analysis in humans. Studies have been performed in human medicine to evaluate the effect of various methods of direct venipuncture, including the influence of differences in size of needle bore and col-

Figure 6—Bland-Altman plot depicting agreement of fibrinogen concentrations in samples collected via 2 methods at time 0. Bias is −1.767 mg/dL, with limits of agreement of −80.639 to 75.973 mg/dL. See Figure 1 for remainder of key.
cation devices; however, no significant differences were detected between the collection methods.

Monitoring coagulation status is an important component of critical care medicine. In humans, numerous methods have been evaluated for obtaining accurate samples from various types of indwelling catheters. Most of the studies have focused on the use of heparinized arterial catheters, which are commonly used for continuous monitoring of blood pressure and for collection of blood samples for laboratory analyses. Although there is considerable variation among techniques, the measurement of APTT is generally less than does the measurement of PT. To obtain valid measurements of APTT, a discard volume of at least 6 times the catheter dead space is considered necessary.

Investigations on the use of venous catheters are more varied. In 1 study, investigators found that there was a significant prolongation in the clotting times for samples collected via a heparinized central venous catheter, compared with results for samples collected via direct venipuncture, and concluded that blood samples for coagulation analysis should be obtained via direct venipuncture. In another study, in which investigators compared collection of samples by use of a heparinized tunneled venous access device with collection of samples via direct venipuncture, it was concluded that there are significant differences in results for coagulation variables, particularly APTT. However, investigators in several other studies conducted to evaluate the accuracy of samples obtained through venous catheters detected no significant differences, especially when the use of heparin was avoided and a sample and discard volume of 2 times the catheter dead space was used.

Less agreement in the measurement of APTT in the study reported here is in keeping with results described for humans. Measurement of APTT would be expected to generate more variability than the measurement of PT because of the longer interval between initiation of the test and the endpoint for APTT. The lowest agreement was found for APTT at 24 hours. This could have been an effect of the APTT being more profoundly influenced than the PT by the activation of hemostatic elements as a result of the presence of the IV catheter; alternatively, it could have represented an effect of the use of heparinized saline solution to flush the catheter during the sample collection technique. However, the amount of volume discarded in the 3-ml syringe technique (3 mL) was greater than 6 times the dead space of the catheter and related T-port (total volume of 0.45 mL). Thus, the 3-mL discard volume is considered adequate for arterial catheters in humans but may not be sufficient for venous catheters in domestic animals. Additional studies to evaluate various discard volumes and to assess samples for heparin contamination may be warranted.

Agreement was adequate for measurements of fibrinogen concentration in the study reported here. Fibrinogen concentration is included in coagulation panels as a modified measurement of thrombin time, which is measured indirectly by the rate of fibrin formation. Interpretation of fibrinogen concentrations in dogs is confounded by the role of fibrinogen as an acute-phase protein. The rate of fibrinogen synthesis by the liver can be massively increased in response to systemic disease. In this study, >50% of the fibrinogen concentrations for samples collected via either method at either time point were mildly or severely increased. This finding was not unexpected, considering that the dogs included in the study were systemically ill and required intensive care.

No attempt was made in this study to assign dogs to groups on the basis of whether their clotting times were within or outside coagulation reference ranges. A cursory examination of the data revealed that 96% of the examined values for PT and APTT for both time points were within the respective reference ranges or were mildly increased. Only 1 dog included in this study had a severe prolongation in both PT and APTT at time 0, which had resolved with treatment by 24 hours. This set of prolonged values was the only datum point that was outside the limits of agreement for the analysis of PT at time 0. On the basis of our results, separate evaluation of agreement between methods for sample collection in animals with coagulopathies should be explored further.

In addition, the study was limited by the time period for which we collected samples. The decision to use a 24-hour interval was based on analysis of the median interval between serial measurements of coagulation variables in hospitalized patients. Some patients will require monitoring of serial coagulation profiles for 48 or 72 hours or longer. On the basis of the data collected at 24 hours, it is likely that agreement at longer intervals may be less than that at 24 hours, especially for measurement of APTT. Additional studies to examine the agreement of PT, APTT, and fibrinogen concentration over prolonged time periods after IV catheter placement are warranted.

Overall agreement of PT between the 2 methods used to obtain blood samples was sufficient to recommend use of samples collected via IV catheter for routine clinical screening and monitoring. Overall agreement of APTT was less than that of PT but was still clinically acceptable. The amount of time that an IV catheter has been in place does not appear to have a significant effect on the measurement of PT, but it may affect measurement of APTT. Avoiding the use of heparin-containing solutions to flush IV catheters and ensuring that a proper discard technique is used when collecting samples may help to provide samples that yield the most reliable results.

References

2. Papp AC, Hatzakis H, Bracey A, et al. ARIC hemostasis study—

a. 16-gauge VenoCath, Abbott Corp, Abbott Ireland, Sligo, Republic of Ireland.
b. AMAX Destiny Plus coagulation analyzer, Trinity Biotech, Carlsbad, Calif.
c. GraphPad Prism 4.0 for Macintosh, GraphPad Software Inc, San Diego, Calif.


