Temporal variation and factors affecting measurement of canine von Willebrand factor

Janet Moser, DVM, PhD; Kenneth M. Meyers, PhD; James H. Meinkoth, DVM, PhD, Jaqueline A. Brassard, DVM, PhD

Objective—To determine whether canine plasma von Willebrand factor (vWf), varies between and within individuals over time and with different blood sample collection and processing procedures.

Animals —26 adult dogs and 6 pups.

Procedure—Blood was obtained from the jugular or cephalic vein daily for 8 to 19 days and weekly for 9 to 23 weeks in adult dogs and periodically up to 180 days of age in pups. Temporal variation in vWf concentration and the effect of vascular occlusion, venipuncture site, lipemia, hemolysis, anticoagulant, storage time, freeze-thawing, and centrifugation speed on plasma vWf concentration, measured by ELISA, were determined.

Results—Plasma vWf concentration varied over time. In dogs with mean vWF concentration ≥79 U/dl, the largest intraindividual range in vWF spanned 64 U/dl with daily and 53 U/dl with weekly sample collection. In dogs with mean vWF concentration ≤24 U/dl, the largest individual variation was 12 U/dl with daily and weekly sample collection. In dogs with mean vWf concentration ≥ 53 and ≤ 74 U/dl, the largest intraindividual range spanned 35 U/dl. Mean vWf concentration of pups from 3 to 180 days of age did not change. Sample hemolysis decreased mean vWf by 37%. Mean vWf concentration was 9% higher in cephalic than jugular vein samples (P = 0.056). Other sample collection/preparation procedures did not affect vWf concentration.

Conclusion—There was substantial temporal variation in vWf concentration within individual dogs.

Clinical Relevance—Multiple tests may be necessary to obtain a reliable estimate of vWf concentration in dogs. *(Am J Vet Res* 1996;57:1288-1293)

Von Willebrand factor (vWf) is an adhesive multimeric glycoprotein that is required for normal hemostasis. On vascular injury, vWf mediates platelet adhesion to exposed subendothelium and is involved, along with fibrinogen, in platelet-to-platelet aggregation.[1] von Willebrand factor also forms a complex with and is a carrier of coagulation factor VIII. The formation of this complex serves to protect factor VIII from proteolytic degradation.[2] In dogs, vWf is produced by vascular endothelial cells and circulates in plasma. Canine platelets contain little to no vWf. von Willebrand factor is the largest protein in plasma, the size of its multimers ranging from 0.5 to 20 million daltons.[3] The large multimers are the most efficient in binding platelets to each other and to exposed subendothelium.

vonWillebrand's disease (vWD) results from a qualitative or quantitative abnormality of vWf. It is the most common hereditary bleeding disorder in dogs and has been described in several breeds.[4] Clinical signs of the disease include spontaneous bleeding from mucosal surfaces and excess blood loss after surgery or trauma. von Willebrand's disease is classified into 3 major categories, types I-III.[5] In type-I vWd, there is a proportionate decrease in all vWf multimers. In type II , vWd, the large multimers are reduced or absent and may be qualitatively abnormal. In type-III vWd, vWf is not detectable. Type-I vWd occurs in many breeds of dogs and is particularly prevalent in Doberman Pinschers, with reported prevalence of 70%.[6] Type-II vWd has been described in German Shorthaired Pointers, and type-III vWd occurs in Scottish Terriers and Chesapeake Bay Retrievers.[6]

In human beings, vWf values may vary with the physiologic state of the individual, making diagnosis of vWd difficult in some instances.[7] In people, vWf concentration increases with advancing age and during exercise, epinephrine stimulation, neurologic stress, pregnancy, and insulin-induced hypoglycemia.[8] Strenuous exercise and epinephrine have also been reported to increase vWf values in dogs. [9] Serial studies[10 11] over short periods indicated large intraindividual variation in vWf concentration in clinically normal people and those with vWd. Because vWf may be repeatedly measured in a dog from samples drawn within days, weeks, or years of each other and vWf concentration has diagnostic and therapeutic importance,[12 13] it is important to know how vWf concentration changes with time.

The manner in which blood samples are collected, processed, and stored can affect the amount of vWf measured. Venostasis for several minutes before blood sample collection causes vWf to increase in human beings,[14 15] possibly by stimulating its release from endothelial intracellular stores. Canine vWf in whole blood increases significantly 24 hours after sample collection and, in plasma, increases 48 hours after acquisition when stored at 20 or 37 C.[16] This increase may be attributable to exposure of additional antigenic sites on breakdown of large multimers into smaller ones.

To the authors knowledge plasma, vWf concentration variations over short periods within an

individual dog, with advancing age in pups, or with various methods of sample collection, and preparation have not been reported. Therefore, baseline plasma vWf values were monitored over time in adult dogs with high vWf concentrations and in adult dogs and pups deficient in vWf. Also assessed were effects on vWf concentration of lipemia, hemolysis, venous occlusion, venipuncture site, various anticoagulants. repeated freezing and thawing of plasma storage time at 20 to 22 C (room temperature) as plasma or whole blood, and different centrifugation speeds.

Materials and Methods

Dogs — Twenty-six adult dogs at least 1 year old and 6 pups up to 6 months old were evaluated. Each dog was categorized as either high (vWf $\geq$ 75 U/dl), mid-range (vWf $\geq$ 30 and < 75 U/dl), low (vWf < 30 and $\geq$ 5 U/dl),[17] or very low (vWf $\leq$ 4 U/dl), according to the mean vWf value measured in multiple samples. Of the 26 adult dogs, 14 were mixed-breed dogs (7 sexually intact and 6 spayed females and 1 neutered male) and 6 were Greyhounds (1 spayed female and 5 neutered males) with high vWf concentrations. Of the remaining adult dogs, 5 were Doberman Pinschers with type-I vWd and low vWf concentrations (3 sexually intact females and 2 sexually intact males) and 1 was a sexually intact female of mixed breeding with very low vWf values, the sire and dam of which had type-I and type-II vWd, respectively. The 6 pups were Doberman Pinscher-types with mid-range vWf values, the dam of which was normal and the sire of which had type-I vWd (4 sexually intact females and 2 sexually intact males). This research was approved by the Animal Care and Use Committee at Washington State University.

Blood sample collection and preparation-Unless otherwise stated, light venous compression was manually applied for approximately 30 seconds during sample collection, and blood was drawn directly into evacuated tubes containing anticoagulant. Blood was anticoagulated either 9:1 in 3.2% trisodium citrate (NaCit) or 100:1 in 15% EDTA. Except as indicated, samples were centrifuged at 1,650 x g at 4 C for 10 minutes within 1 hour of collection. The plasma was transferred to plastic vials and stored frozen at -20 C until assayed.

Intraindividual variation, venipuncture site, and variation over time - To determine intraindividual variation in vWf concentration, blood was obtained via jugular or cephalic venipuncture every 24 hours for 8 to 19 days and weekly for 9 to 23 days at approximately the same time of day. Six sexually intact mixed-breed female dogs, 5 Doberman Pinschers (3 females and 2 males with type-I vWd) and the mixed-breed female with very low vWf values were tested. Blood was drawn from the jugular vein of 6 pups at 3, 10, 20, 30, 45, 60, and 180 days of age. Samples were anticoagulated with EDTA or NaCit.

Lipemia and hemolysis - To assess whether vWf concentration is affected lipemia or hemolysis, blood was drawn from the cephalic vein into 2 tubes containing NaCit from 7 mixed-breed dogs (6 spayed females and 1 neutered male) after a 12-hour nonfeeding period. The dogs were then fed a high-fat (2 females and 1 male) or a low-fat (4 females) diet, and 2 hours after eating, a second blood sample was obtained and anticoagulated with NaCit. Lepemia was assessed visually and graded as nil, light, or heavy. To create hemolysis, 1 sample obtained from each dog at the end of the nonfeeding period was frozen at -20 C for 8 hours, then thawed in a 37 C water bath. One sample was unintentionally hemolyzed during collection.

Collection techniques and anticoagulants - Six Greyhounds were used to examine whether collection techniques or anticoagulant influence vWf values. For each dog, blood was drawn from occluded right jugular and right cephalic veins and from nonoccluded left jugular and left cephalic veins. Samples were collected in duplicate from each of the 4 veins. One sample from each vein was anticoagulated with EDTA, and the other sample was anticoagulated with NaCit.

Sample freezing - To determine the effect of repeated freezing and thawing of plasma on vWf concentration, samples were drawn on 11 days from the cephalic vein of 1 mixed-breed sexually intact female dog, using EDTA as the anticoagulant. Each sample underwent 4 freeze-thaw cycles (frozen at -20 C) and, after each thawing, vWf was measured.

Sample storage time - Blood was obtained from 4 Greyhounds to test the effect on vWf concentration of storage time as plasma or whole blood. Blood was drawn from the jugular vein into 5 tubes containing EDTA and 5 tubes containing NaCit. To determine the effect of storage time as plasma, blood from 2 of the dogs (1 female and 1 male) was centrifuged within 10 minutes of collection. Immediately and 2, 4, 6, and 8 hours after centrifugation, plasma from 1 tube containing EDTA and 1 tube containing NaCit from each dog was removed from the tubes and frozen; prior to freezing, the samples were stored at 20 to 22 C (room temperature). To determine the effect of storage time as whole blood, 1 sample with EDTA and 1 sample with NaCit from each of the other 2 Greyhounds (both males) were centrifuged within 10 minutes of collection and 2, 4, 6, and 8 hours after standing at 20 to 22 C. Plasma was frozen immediately after centrifugation.

Centrifugation effects - To assess the effect of centrifugation speed on vWf values, blood was collected from the jugular vein into 3 tubes containing EDTA and 3 tubes containing NaCit from each of 2 male Greyhounds. Blood from 1 of the Greyhounds was inadvertently hemolyzed in the tubes containing EDTA; samples were therefore, drawn from 1 sexually intact mixed-breed female dog with a high vWf concentration, and the experiment, using EDTA as the anticoagulant, was repeated. Blood in 1 tube containing EDTA and 1 tube containing NaCit from each dog was centrifuged at 100, 400, or 1,650 x g at 20 C. Blood was centrifuged at 20 C to avoid loss of platelet viability attributable to chilling. [18] Immediately after centrifugation, the plasma was transferred to plastic tubes. Platelets and erythrocytes in the plasma were counted within 2 hours of sample collection, using a microcollection system,[19.a] and the samples were then frozen.

von Willebrand factor assay - Plasma vWf concentration was measured in triplicate by use of a modified capture ELISA. A rabbit polyclonal antibody specifically directed against canine vWf was prepared as described, using vWf isolated by gel filtration from cryoprecipitates.[20 21] This antibody reacts with all sizes of vWf multimers and reflects changes that occur in the multimeric pattern. Ninety-six-well microtitration plates[b] were coated with 50 µl of this antibody/well (diluted 1:1,000 in 15 m*M* Na_2CO_3, 34.9 m*M* $NaHCO_3$). The plate was covered, incubated overnight at 4 C, then washed 6 times with phosphate-buffered saline solution (PBSS), which consisted of 8.8 m*M* dibasic $NaPO_4$ 2.25 m*M* monobasic $NaPO_4$, and 151 m*M* NaCl (pH 7.4). The antibody-coated wells were blocked with 180 υl of 3% bovine serum albumin in PBSS (PBSSA)/well for 30 minutes at 37 C. The PBSSA was then removed by inverting the plate.

Antigen was either the sample to be measured or dilutions of normal pooled plasma for construction of the standard curve. Samples and standards were diluted with 1% PBSSA.

Table 1 —Plasma von Willebrand factor (vWf) concentrations in samples drawn over consecutive days from 6 mixed-breed dogs with high vWf values and 6 dogs with low or very low vWf values

Dog group	Mean vWf (U/dl)	Range (U/dl)	95% Ci for the mean (U/dl)	No. of daily samples
Mixed breed				
1	88	79-106	± 5	12
2	125	88-152	±10	13
3	113	93-144	±10	13
4	79	74-91	± 3	13
5	79	71-93	± 3	13
6	143	118-172	± 9	13
Type-I vWd				
1	24	22-30	± 1	19
2	16	13-19	± 1	17
3	18	15-22	± 1	18
4	16	12-23	± 2	13
5	20	12-24	± 2	17
Mixed-type vWd				
1	2	1-3	± 1	8

Ci = confidence interval; vWf = von Willebrand's disease

Table 2 —Plasma vWf concentrations in samples drawn over consecutive weeks from 6 mixed-breed dogs with high vWf values and 6 dogs with low or very low vWf values

Dog group	Mean vWf (U/dl)	Range (U/dl)	95% Ci for the mean (U/dl)	No. of daily samples
Mixed breed				
1	85	75-102	± 7	10
2	122	91-142	±11	11
3	108	90-125	±7	13
4	83	69-122	± 10	10
5	85	71-101	± 7	13
6	139	118-171	± 10	12
Type-I vWd				
1	23	16-28	± 2	19
2	16	13-19	± 1	16
3	17	12-22	± 1	18
4	18	12-24	± 2	15
5	19	13-24	± 1	23
Mixed-type vWd				
1	4	2-7	± 2	9

Samples were diluted 1:50 to 1"250 so that assay results would be near the middle of the standard curve. Twenty-five microliters of antigen was added to each well, and the plate was incubated for 2 hours at 37 C. After 6 washes with phosphate-buffered Tween (0.05%), the wells were again blocked with 3% PBSSA for 30 minutes at 37 C. The PBSSA was then removed.

There is good cross-reactivity between canine vWf and antibodies produced against human vWf.[22] Captured vWf was, therefore, detected by adding 50 υl of a 1:200 dilution of rabbit anti-human vWf antibody coupled with horseradish peroxidase[c]/well. The mixture was incubated for 2 hours at 37 C. The antibody bound to the remaining free antigenic determinants of vWf. After incubation, the plates were washed 6 times with PBSS.

Bound peroxidase was revealed by its activity on the substrate *o*-phenylenediamine[d] in the presence of hydrogen peroxide. Four milligrams of *o*-phenylenediamine and 5 υl of 30% H^20^2 were dissolved in 10 ml of citrate phosphate and 50 µl of this solution was then added to each well. Color development was stopped after a 5-minute room temperature incubation by addition of 50 µl of 1*N* HCl/well. Absorbance was read at 492 nm, using a microtitration plate scanner.[e] To standardize the samples anticoagulated in EDTA with those anticoagulated in NaCit, data were corrected to adjust for NaCit dilution.

Validation of the ELISA - The standard curve for quantification of canine vWf was constructed by serially diluting pooled canine plasma obtained from combining equal volumes of plasma, from samples anticoagulated in EDTA, from 20 clinically normal adult dogs. After logarithmically transforming absorbance readings and vWf concentrations, the standard curve was consistently linear (R^2 = 0.997 ± 0.002 [SD] for 20 standard curves) over a dilution range of 1:100 to 1:1,000. Normal pooled plasma was arbitrarily assigned to contain 100 U of vWf/dl, and vWf in plasma samples was expressed in units that were a percentage of vWf, compared with that in normal pooled plasma. The lower detection limit of the assay was 2.1 times the mean absorbance value of the blank and corresponded to a mean (± SD) vWf concentration of 0.098 ± 0.004 U/dl (measured in quadruplet in 20 experiments).

Intra-assay variation was assessed by using plasma from 4 dogs, the vWf values of which ranged from very low to normal. There were 11 replications/sample/plate; each replication was assayed in triplicate. The intra-assay coefficient of variation was 8.1% for normal (mean, 112 U/dl), 6.4% for mid-range (mean, 52 U/dl), 6.2% for low (mean, 19 U/dl), and 5.9% for very low (mean, 5 U/dl) vWf concentrations.

The interassay coefficient of variation was 11.8%. This was determined from triplicate vWf measurements on each of 20 plates in plasma with mean vWf concentration of 13 U/dl.

Specificity of the assay was confirmed by use of vWf-deficient plasma from a Scottish Terrier with type-III vWd.[f] Absorbance values were not higher than the blank value in undiluted and diluted (1:10 to 1:1,000) plasma, indicating that the assay was monospecific for canine vWf.

Data analysis - To determine whether vWf concentration changed significantly over time in adult dogs, multiple regression analysis was performed and between-subjects variability was allowed by use of dummy variables treated with effects coding. [23] Multiple regression, using dummy variables and effects coding, also was performed to analyze the effect of venipuncture site on vWf concentration when several samples were drawn at different times from each of the cephalic and jugular veins. One-way ANOVA for repeated measures was performed to determine whether vWf concentration significantly changed over time within pups, over storage time as whole blood or plasma, with repeated freezing and thawing of samples, or with variable centrifugation speed. One-way repeated measures ANOVA also was used to assess whether platelet count varied with different centrifugation speeds. Three-way repeated measures ANOVA was performed to determine whether vWf concentration was significantly affected by venous occlusion, anticoagulant used, or venipuncture site when samples were drawn once from the cephalic and jugular veins. When a significant difference was found, Tukey's multiple comparison method was used to identify pairwise differences. To determine whether there were significant differences in vWf concentration before and after lipemia or hemolysis, a paired t-test was performed. Differences were considered significant at $P < 0.05$.

Results

Individual variability in vWf concentration - Mean plasma vWf concentration in the 6 mixed-breed female dogs after daily sample collection was $\geq$ 79 U/dl (Table 1); plasma concentration of vWf in the daily samples from individual dogs varied considerably. The largest range was 88 to 152 U/dl in dog 2. There also was considerable variation for weekly sample collection (Table 2). The largest range was 118 to 171 U/dl in dog 6.

Mean plasma vWf concentration in 5 Doberman Pinschers with type-I vWd was 16 to 24 U/dl for daily and weekly samples. The largest individual variation was 12 U/dl in dog 5 (Table 1) and dogs 1 and 4 (Table 2).

Mean plasma vWf concentration in the 6 pups tested 7 times from 3 to 180 days of age was 53 to 74 U/dl (Table 3). The largest variation in vWf concentration was in pup 6, values for which ranged from 42 to 77 U/dl. Individual variation in vWf concentration in mixed-breed dogs, dogs with type-I vWd, and pups appeared random without a trend or pattern.

von Willebrand factor concentration in individual samples from adult mixed-breed dogs and pups overlapped. Concentration of vWf in the adult mixed-breed dogs ranged from 69 to 172 U/dl (Tables 1 and 2);

Table 3 — Plasma vWf concentrations in samples drawn periodically up to 180 days of age from 6 pups

Pup No.	Mean vWf (U/dl)	Range (U/dl)	95% Ci for the mean (U/dl)	No. of daily samples
1	53	37-62	± 8	7
2	62	45-71	±10	7
3	74	64-80	± 5	7
4	66	62-71	± 3	7
5	56	46-66	± 7	7
6	59	42-77	± 12	7

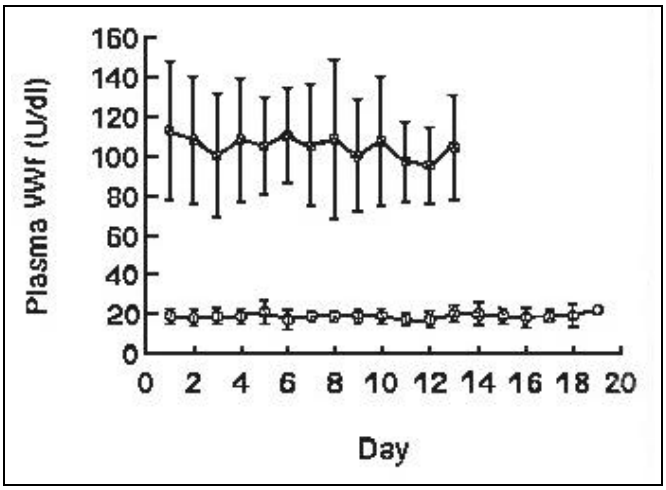

Figure 1—Plasma von Willebrand factor(vWD) concentration over time in samples drawn daily from 6 dogs with high vWf (■) and 5 dogs with low vWf (○) values. The lines were plotted from data in Table 1. Number of sample collection days varied from 12 to 13 for dogs with high vWf values and 13 to 19 for dogs with low vWf values. One dog was tested for 19 days. Data are mean ± SD plasma vWf concentration.

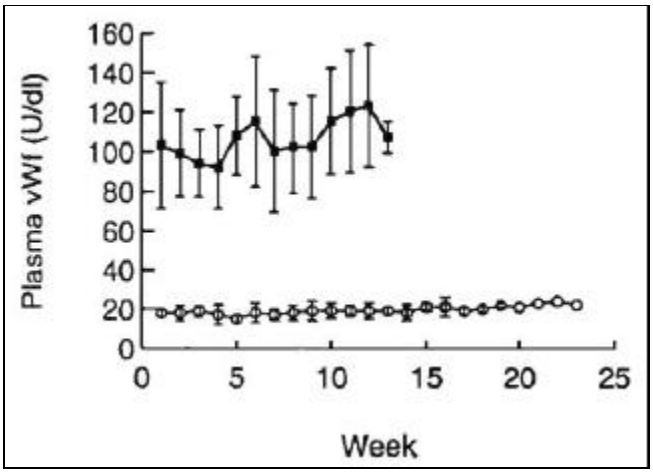

Figure 2 —Plasma vWf concentration over time in samples drawn weekly from 6 dogs with high vWf (■) and 5 dogs with low vWf (○) values. The lines were plotted from data in Table 2. Number of sample collection weeks varied from 10 to 13 for dogs with high vWf values and 15 to 23 for dogs with low vWf values. One dog was tested for more than 19 weeks. See Figure 1 for key.

vWf concentration in samples from the pups ranged from 37 to 80 U/dl (Table 3). There was no overlap in plasma vWf concentration between pups and dogs with type-I vWd.

Variability in vWf values over time - von Willebrand factor concentrations over time after daily sample collection for dogs with high and low vWf values were compared (Fig. 1). Multiple regression analysis of the data indicates that vWf concentration did not change over the daily sample collection period in dogs with low vWf values ($P = 0.14$, $R^2 = 0.65$). In dogs with high vWf values, there was a slight negative trend in vWf values for daily sample collection ($P = 0.01$, $R^2 = 0.83$); vWf concentration decreased by an average of 0.921 U/dl/d.

von Willebrand factor concentrations over time for weekly sample collection in dogs with high and low vWf values were evaluated (Fig. 2). Multiple regression analysis suggested a slight negative trend in vWf concentration over the weekly sample collection period in dogs with low vWf values; vWf decreased by an average of 0.173 U/dl/wk ($P = 0.001$, $R^2 = 0.48$). In dogs with high vWf values, vWf concentration increased slightly with weekly sample collection by an average of 1.31 U/dl/wk ($P = 0.006$, $R^2 = 0.77$). Analysis of variance of vWf concentration in pups with mid-range vWf values indicates that mean vWf concentration did not change significantly at any time during the first 180 days of life (Fig. 3; $P = 0.08$).

Variability in vWf values attributable to sample collection technique and sample preparation - Sample hemolysis significantly decreased vWf values. Mean vWf concentration in the 6 samples hemolyzed by freezing was 37% less than that in the corresponding nonhemolyzed samples (89 $\pm$ 35 U/dl vs 141 $\pm$ 52 U/dl, P = 0.003). von Willebrand factor concentration in the sample that was unintentionally hemolyzed during collection was 88% lower than its nonhemolyzed counterpart (171 U/dl vs 21 U/dl).

von Willebrand factor concentration inconsistently changed with repeated freezing and thawing in the 11 samples studied (Table 4; $P = 0.0002$). Mean vWf concentration after 1 thawing did not differ significantly from mean vWf concentration after 3 or 4 thawings, but after 1 thawing, the mean value was significantly higher than that after 2 thawings.

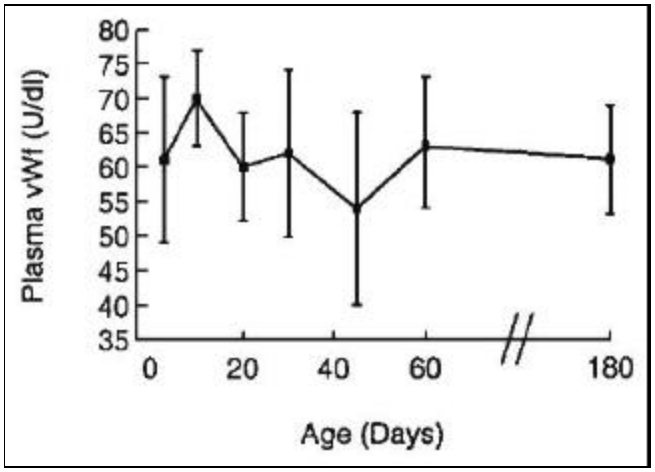

Figure 3 —Plasma vWf concentration over time in 6 pups with mid-range vWf values. The line was plotted from data in Table 3. Seven samples were drawn from each dog during the first 180 days of life. See Figure 1 for key.

Table 4 - Effect on vWf concentration of repeated freezing and thawing of canine plasma

Thawings	Plasma vWf (U/dl)
1	$118 \pm 8^{a.b}$
2	101 ± 12^{c}
3	$107 \pm 19^{b.c}$
4	120 ± 20^{a}

Values with different superscripts are significantly different from each other, $P<0.05$. Data are mean $\pm$ SD for 11 plasma samples.

In samples drawn at several times from each of the cephalic and jugular veins, plasma vWf concentration did not vary between the 2 veins, as indicated by multiple regression analysis. (Over daily sample collection: $P = 0.24$, $R^2 = 0.83$ in dogs with high vWf concentration; $P = 0.50$, $R^2 = 0.65$ in dogs with low vWf concentration, and over weekly sample collection: $P = 0.66$, $R^2 = 0.77$ in dogs with high vWf concentration; $P = 0.35$, $R^2 = 0.49$ in dogs with low vWf concentration.) However, the difference between mean vWf values in samples drawn once from each cephalic and jugular vein was of borderline significance. Mean vWf concentration in plasma obtained once from the cephalic vein was 9% higher than that in plasma obtained once from the jugular vein (118$\pm$ 26 U/dl vs 108 $\pm$ 26 U/dl in 6 dogs, $P = 0.056$).

von Willebrand factor concentration was not significantly affected by lipemia (data not shown; $P = 0.55$ in 4 dogs and $P = 0.54$ in 3 dogs with light and heavy lipemia, respectively). There also was no difference in vWf concentration between samples drawn from an occluded versus a nonoccluded vein ($P = 0.43$) or between use of NaCit and EDTA as anticoagulants)$P = 0.55$) in 6 dogs. Storage at room temperature for up to 8 hours did not significantly change vWf values in plasma or whole blood when NaCit or EDTA were used as anticoagulants (data not shown; for plasma: $P = 0.52$ with NaCit, P = 0.44 with EDTA in 2 dogs; for whole blood: P = 0.17 with NaCit, $P = 0.76$ with EDTA in 2 dogs). von Willebrand factor concentration did not significantly differ among the 3 centrifugation speeds tested (100, 400, and 1,650 x g), using NaCit or EDTA as anticoagulants (data not shown; P = 0.52 with NaCit, $P = 0.53$ with EDTA in 2 dogs). Numbers of erythrocytes in plasma after centrifugation at all 3 speeds were at or below detection (10,000 cells/ul) in all tubes. Platelet count in plasma from blood anticoagulated with NaCit was significantly ($P = 0.001$) higher when centrifuged at 100 x g than when centrifuged at the other 2 speeds. Platelet count in plasma from blood anticoagulated with EDTA did not differ significantly with centrifugation speed.

Discussion

There was considerable individual variation in plasma vWf concentration in blood samples drawn daily or weekly from an individual dog. In dogs with low vWf values (mean, $\geq$ 16 to $\leq$ U/dl), plasma vWf concentration did not increase above 30 U/dl. There was no overlap between values for these dogs and for the pups with mid-range vWf concentration (mean, 53 to 74 U/dl) or for mixed-breed dogs with high vWf concentration (mean, > 79 U/dl). Mid-range vWf concentrations varied between 37 and 80 U/dl and overlapped with high vWf concentrations. von Willebrand factor increased to high values in 4 of the 6 dogs with mid-range concentration and decreased to mid-range in 3 of the 6 dogs with high vWf values. These observations may have clinical relevance. Dogs with mid-range vWf concentration, as measured by electroimmunoassay and ELISA,[17] have been suggested to be carriers of vWd.[4] The individual variation reported in this study points out the difficulty in attempting to establish the genotype from a single vWf measurement.

Slight daily and weekly statistically significant changes in vWf concentration were observed, using multiple regression analysis with dummy variables. Dummy variables were used to decrease the effect of variability between dogs, but their use makes multiple regression analysis powerful and sensitive to small changes. Therefore, these slight changes in vWf values over days and weeks may not have biological relevance.

von Willebrand factor concentration does not change significantly in pups between the ages of 3 and 180 days. Testing for vWd in young pups should, therefore, produce similar results as testing at 6 months of age. Other investigators found changes in vWf concentration of pups to be irregular over time.[24]

The method of sample collection and processing can also influence vWf concentration. Sample hemolysis results in a significant reduction of measurable vWf. This result is in agreement with the findings of others who measured canine vWf concentration in hemolyzed and nonhemolyzed samples, using rocket immunoelectrophoresis.[25]

Mechanical injury to and subsequent release of vWf from the endothelium could result in high plasma vWf values. The cephalic vein is smaller than the jugular vein and, consequently, is more susceptible to trauma during blood sample collection. Slight increases in single samples, but not repeat samples, suggest there could be a slight but not major increase in vWf concentration in blood samples collected from the cephalic vein.

Multiple freezing and thawing of plasma at least 4 times has no effect on vWf concentration. Similar results were obtained in studies in which canine vWf concentration was measured by use of electroimmunoassay[26] and by an ELISA.[27] The inconsistent changes observed in this study are most likely attributable to chance because vWf values after 1 and 4 thawings did not differ.

Canine blood samples, anticoagulated with NaCit or EDTA, can be kept at room temperature for at least 8 hours as plasma or whole blood and can be centrifuged at speeds at least as low as 100 x g without affecting vWf concentration. The platelet count is significantly higher in canine plasma after centrifuging blood at 100 x g than after centrifugation at higher speeds, but unlike platelets of other species, canine platelets contain negligible amounts of vWf,[28] and platelet presence should not alter the vWf value. However, low-speed centrifugation of blood from species other than the dog may result in increased plasma vWf concentration owing to the release of vWf from platelets in the plasma sample.

Venous occlusion for approximately 30 seconds does not influence canine vWf concentration. In human beings, venostasis for several minutes before blood sample collection causes vWf values to increase.[14 15] Values for fibronectin and tissue plasminogen activator, 2 other endothelial cell-derived proteins involved in hemostasis, also increase in plasma after prolonged venous occlusion in human beings.[29 30]

Canine vWf concentration is not affected by lipemia. There also is no difference in vWf values between either NaCit or EDTA used as anticoagulants.

In conclusion, plasma vWf concentration varies daily and weekly within an individual dog. von Willebrand factor values do not change in pups with mid-range values during the first 180 days of life. Sample hemolysis decreases vWf concentration in canine plasma; for reliable results, vWf should be measured in nonhemolyzed plasma samples. Blood may be lipemic, collected from occluded or nonoccluded jugular veins, frozen and thawed as plasma at least 4 times, anticoagulated with either NaCit or EDTA, centrifuged at speeds at least as low as 100 x g. and stored at room temperature as plasma or whole blood for at least 8 hours without affecting vWf values.

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[a]Unopette, Becton-Dickinson Co. Rutherford, NJ.
[b]Costar, Cambridge, Mass.
[c]Dako Corp, Carpenteria, Calif.
[d]Sigma Chemical Co, St. Louis, Mo.
[e]Titertek multiscan MCC/340, Flow Laboratories, McLean, Va.
[f]Courtesy of Dr. W. J. Dodds, Santa Monica, Calif.

References

1. Ruggeri ZM, Ware J. The structure and function of von Willebrand factor. *Thromb Haemost* 1992;67:594-599.

2. Lollar P. The association of factor VIII with von Willebrand factor. *Mayo Clin Proc* 1991;66:524-534.

3. Wagner DD, Bonfanti R. von Willebrand factor and the endothelium. *Mayo Clin Proc* 1991;66:621-627.

4. Johnson GS, Turrentine MA, Kraus KH. Canine von Willebrand's disease. *Vet Clin North Am Small Anim Pract* 1988;18:195-229.

5. Sadler JE, Gralnick HR. Commentary: a new classification for von Willebrand disease. *Blood* 1994;84:676-679.

6. Dodds WJ. Contributions and future directions of hemostasis research. *J Am Vet Med Assoc* 1988;193:1157-1160.

7. Blomback M, Eneroth P, Andersson O. et al. On laboratory problems in diagnosing mild von Willebrand's disease. *Am J Hematol* 1992;40:117-120.

8. Bloom AL. von Willebrand factor: clinical features of inherited and acquired disorders. *May Clin Proc* 1991;66:743-751.

9. Meyers KM, Wardrop KJ, Dodds WJ. et al. Effect of exercise, DDAVP, and epinephrine on the factor VIII:C., von Willebrand factor complex in normal dogs and von Willebrand factor deficient Doberman Pinscher dogs. *Thromb Res* 1990;57:97-108.

10. Blomback M, Eneroth P, Landgren BM, et al. On the intraindividual and gender variability of haemostatic components. *Thromb Haemost* 1992;67:70-75.

11. Abildgaard CF, Suzuki Z, Harrison J, et al. Serial studies in von Willebrand's disease: variability versus "variants". *Blood* 1980;56:712-716.

12. Meyers KM, Wardrop KJ, Meinkoth J. Canine von Willebrand's disease: pathobiology, diagnosis, and short-term treatment. *Compend Contin Educ Pract Vet* 1992;14:13-22.

13. Ching YNLH, Meyers KM, Brassard JA, et al. Effect of cryoprecipitate and plasma on plasma von Willebrand factor multimers and bleeding time in Doberman Pinschers with type-I von Willebrand's disease. *Am J Vet Rcs* 1994;55:102-110.

14. Ponari O, Pini M, Poli T, et al. Correlation between changes induced by venous occlusion on factor VIII-von Willebrand factor components and fibrinolytic activity. *Haemostatsis* 1984;14:179-183.

15. Blann AD, Wainwright AC, Sheeran TP, et al. Venostasis, subclinical vasculitis, and von Willebrand factor antigen. *Br J Rheumatol* 1991;30:373-375.

16. Mansell PD, Parry BW. Stability of canine factor VIII activity and von Willebrand factor antigen concentration in vitro. *Res Vet Sci* 1991;51:313-316.

17. Moser J, Meyers KM, Russon RH. Inheritance of von Willebrand factor deficiency in Doberman Pinschers. *J Am Vet Med Assoc* 1996 in press.

18. Slichter SJ, Harker LA, Preparation and storage of platelet concentrates. II. Storage variables influencing platelet viability and function. *Br J Haematol* 1976;34:403-419.

19. *Laboratory procedures using the Unopette brand system.* 8th ed. Rutherford, NJ: Becton-Dickinson Co. 1977;12,16.

20. Zimmerman TS, Roberts JR, Factor-VIII-related antigen. In: Nokamura RM, Dito WR. Tucker ES, eds. *Immunoassays: clinical laboratory techniques for the 1980's.* New York: Alan R Liss Inc, 1980;339-349.

21. Meyers KM, Wardrop KJ, Helmick C, et al. von Willebrand factor is present in the vascular endothelium from normal dogs and from Doberman Pinscher dogs with a plasma von Willebrand factor deficiency. *Thromb Res* 1990;57:109-116.

22. McCarroll DR, Lothrop SA, Dolan MC, et al. Canine von Willebrand factor expresses a multimeric composition similar to human von Willebrand factor. *Exp Hematol* 1987;15:1060-1067.

23. Glantz SA, Slinker BK. Repeated measures. In: *Primer of applied regression and analysis of variance.* New York: McGraw-Hill Book Co, 1990-381-460.

24. Mansell PD, Parry BW. Changes in factor VIII activity and von Willebrand factor antigen concentration with age in dogs. *Br Vet J* 1992;148:329-337.

25. O'Neill SL, Feldman BF. Hemolysis as a factor in clinical chemistry and hematology of the dog. *Vet Clin Pathol* 1989;18:58-68.

26. Benson RE, Jones DW, Dodds WJ. Efficiency and precision of electroimmunoassay for canine factor VIII-related antigen. *Am J Vet Res* 1983;44:399-403.

27. Benson RE, Catalfamo JL, Brooks M, et al. A sensitive immunoassay for von Willebrand factor. *J Immunoassay* 1991;12:371-390.

28. Parker MT, Turrentine MA, Johnson GS. von Willebrand factor in lysates of washed canine platelets. *Am J Vet Res* 1991;52:119-125.

29. Letowska M, Bykowska K, Sablinski J, et al. Venostasis but not DDAVP infusion provokes the plasma fibronectin increase. *Thromb Haemost* 1990;64:294-296.

30. Nilsson IM, Vilhardt H, Holmberg L, et al. Association between factor VIII related antigen and plasminogen activator. *Acta Med Scand* 1982;211:105-12.

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From the Departments of Pharmacology/Toxicology (Moser) and Veterinary and Comparative Anatomy, Pharmacology, and Physiology (Meyers, Meinkoth, Brassard), Washington State University, Pullman, WA 99164-6520. Dr. Moser's present address is the United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425. Dr. Meinkoth's present address is Veterinary Pathology, Oklahoma State University, 250 Vet Med, Stillwater, OK 74078-2007. Dr. Brassard's present address is Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92038.

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