Assessment of changes in hemostatic markers in Cavalier King Charles Spaniels with myxomatous mitral valve disease

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Objective—To evaluate markers of hemostasis and their relationship to the degree of mitral regurgitation (MR) and platelet function in Cavalier King Charles Spaniels (CKCSs) with myxomatous mitral valve disease.

Animals—76 clinically healthy CKCSs and 24 control dogs.

Procedure—All dogs underwent echocardiographic examination; various hemostatic, hematologic, and biochemical variables were evaluated in blood. The CKCSs were allocated to 1 of 3 groups on the basis of MR severity. In 8 control dogs and 8 CKCSs, plasma von Willebrand factor (vWF) multimer analysis was performed.

Results—Compared with control dogs, plasma fibrinogen concentration was higher in all CKCSs and related to left ventricular end diastolic diameter and left atrial-to-aortic root ratio among all CKCSs. The activated partial thromboplastin times and plasma D-dimer concentration were similar among the 4 groups. Plasma vWF concentration was lower in CKCSs with moderate to severe MR, compared with that of CKCSs with no MR and control dogs. There was a relationship between plasma vWF concentration and platelet function in CKCSs but not in control dogs. In 4 CKCSs with moderate to severe MR and low plasma vWF concentration, amounts of vWF high-molecular-weight multimers (HMWMs) were low.

Conclusions and Clinical Relevance—In CKCSs, MR appeared to be associated with a low plasma vWF concentration and likely a loss of vWF HMWMs (possibly through their destruction via shear stress to the blood). The importance of the changes in plasma fibrinogen concentration and the thromboembolic risk in dogs with MR remain to be investigated. (Am J Vet Res 2004;65:1644–1652)

Myxomatous mitral valve disease (MMVD) is the most prevalent heart disease in dogs. It is characterized by a myxomatous degeneration of the valve that may result in substantial mitral regurgitation (MR) in some, but not all, dogs. Mitral valve prolapse (MVP) is a fundamental feature of MMVD; in many ways, MVP in dogs is similar to primary MVP in humans. Cavalier King Charles Spaniels (CKCSs) are predisposed to MMVD, and most CKCSs develop a systolic murmur as a result of MR before they reach 10 years of age.

In dogs, changes in the hemostatic system have been reported in association with disease syndromes such as parvoviral enteritis, gastric dilatation-volvulus, and hemangiosarcoma. Many humans with cardiovascular disease have hypercoagulation, and the changes in the coagulation system are often associated with atherosclerosis. In cardiovascular abnormalities in which atherosclerosis is not regarded as an underlying feature, such as primary MVP and aortic valve disease, hypercoagulability and increased risk of stroke have been reported. Because of the diseased cardiac valves, regions of increased turbulence and shear stress develop that can activate platelets and coagulation factors in the blood and damage the vascular endothelium.

The process of normal physiological hemostasis is dependent on collaboration between the hemostatic system and the endothelium, which maintains vascular integrity and normal vascular function. Changes in markers of hemostatic dysfunction would therefore theoretically be accompanied by a change in markers of endothelial dysfunction. Recently, a decreased plasma concentration of nitric oxide metabolites was identified in CKCSs with MR, compared with the concentration in clinically normal dogs without MR, which indicates that dogs with MR have endothelial dysfunction; therefore, other and more easily measured markers of endothelial dysfunction would be relevant to study in dogs with MR. Compared with clinically normal individuals, an increase in plasma von Willebrand factor (vWF) concentration has been shown to be a consistent marker of endothelial dysfunction in humans and vWF concentration is one of the few endothelial dysfunction markers that can be measured readily in canine plasma. Fibrinogen is a clotting fac-
Materials and Methods

In CKCSs, an increased fibrinolytic activity and a marker of increased fibrin production or consumption were identified in dogs with MMVD and in CKCSs in particular because this breed also has a high prevalence of inherited macrothrombocytopenia. In CKCSs, an increased platelet reactivity has been detected in dogs without macrothrombocytopenia. In addition, a decreased platelet function has been identified in dogs with moderate to severe MR. Thus, it has been speculated that platelet function plays a role in the pathogenesis of MMVD and MR. Because platelets are intimately linked to the coagulation system, platelet function should be considered in studies of the coagulation system.

The purpose of the study reported here was to evaluate markers of hemostasis and their relationship to the degree of MR and to plateau count and function in CKCSs with MMVD. We hypothesized that CKCSs with MMVD may have endothelial dysfunction and alterations in markers of hemostasis, compared with healthy dogs, and that such changes might perhaps reflect hemodynamic effects and shear stress associated with MR.

Materials and Methods

Animals—Seventy-six client-owned CKCSs (median age, 4.3 years; 25th to 75th percentiles, 2.3 to 6.0 years) with no clinical signs associated with MR and 24 client-owned clinically normal control dogs (median age, 3.9 years; 25th to 75th percentiles, 2.4 to 5.4 years) were included in the study. Among the CKCSs, there were 38 males (2 of which were neutered) and 38 females; among the control dogs, there were 14 males (3 of which were neutered) and 10 females (1 of which was neutered). The CKCSs underwent cardiac examination consecutively from January 2002 to February 2003 as part of an MMVD screening program to be approved by the owners of all dogs. Blood sample collection and handling—Blood was collected by the same investigator (HDP) with careful venipuncture of the jugular vein into tubes containing no anticoagulant, 3.2% sodium citrate, or EDTA. For each dog, serum was used for biochemical analyses; the citrated blood sample was used for platelet function analysis (whole blood), a hemostatic profile (APTT, PT, and fibrinogen and D-dimer concentrations), measurement of vWF concentration, and multimer analysis (plasma); and blood with EDTA and a manual count of platelets. Specimens of plasma and serum were prepared by centrifugation at 3,100 × g for 10 minutes within 1 hour of blood collection and portioned into aliquots. Sera were stored at –80°C until analysis.

Analytical procedures—A CBC and serum biochemical analyses were performed for each dog. The hemostatic profile (including assessment of APTT, PT, and plasma fibrinogen and D-dimer concentrations) and plasma vWF concentration were assessed by use of an automated chemical analyzer. Assessment of the APTT involved the use of synthetic phospholipids and silica to trigger coagulation. The PT and fibrinogen concentration were evaluated simultaneously by use of a rabbit brain calcium thromboplastin. Light scattering before and after clot formation was recorded by use of the automated chemical analyzer, and the fibrinogen concentration was calculated by use of these values and a calibration curve. Concentrations of D-dimer and vWF were measured via a turbidometric immunoassay. A pooled sample of plasma from 13 to 15 clinically normal dogs without evidence of bleeding was analyzed together with the samples. The D-dimer concentration was only determined in 8 control dogs and 43 CKCSs (consecutively examined during the period January 2002 to June 2002) because preliminary results indicated that there was no difference in D-dimer concentration between control dogs and CKCSs. Plasma samples were thawed at 37°C in a waterbath immediately before analysis and centrifuged at 3,000 × g for 5 minutes (to avoid remnants of cryoprecipitate in plasma after thawing); the supernatants were used for analysis.

Manual counts of platelets were performed by use of a stromatolytic agent and a counting chamber, as previously described. The fixed platelets were counted in duplicate on the day of the examination. A platelet function analyzer was used to assess platelet function according to the manufacturer’s instructions; this device has been described in detail. In brief, the platelet function analyzer measures platelet function in vitro by aspirating citrated whole blood from a test cartridge through a 150-μm-diameter aperture in a membrane coated with a combination of collagen and adenosine diphosphate, and the platelet aggregation is measured. A platelet plug closes the aperture in the membrane, and the instrument monitors the time required for complete occlusion (closure time measured in seconds). The cutoff time of the instrument is 300 seconds (ie, nonclosure is reported if the
and the diluted sample was heated for 20 minutes at 60 °C. A mophenol blue; pH, 8.0); 350 µL of the diluted sample was used in an agarose gel electrophoresis (SDS-AGE), western blot analysis, and immunoperoxidase detection. The technique has been described in detail.27 In brief, SDS-AGE was performed in a 1% separating gel prepared by use of high-gelling-temperature agarose dissolved in gel buffer. electrophoresis was performed in electrophoresis buffer (0.05M Tris, 0.394M glycine, and 0.1% SDS; pH, 8.4) at a constant current of 12.5 mA/gel (duration, approx 16 hours). Resolved protein was transferred to a polyvinylidene fluoride membrane via electrotransfer at 33 V for 6.5 hours (2 gels). After transfer, the polyvinylidene fluoride membrane was blocked in 0.5% bovine serum albumin diluted in 0.1M PBS (0.01M Tris, 1mM EDTA-Na2, 8M urea, 2% SDS, and bro-mophenol blue; pH, 8.0); 350 µL of stacking gel was added, and the diluted sample was heated for 20 minutes at 60°C. A 40-µL volume was pipetted into each well, and horizontal electrophoresis was performed in electrophoresis buffer (0.05M Tris, 0.394M glycine, and 0.1% SDS; pH, 8.4) at a constant current of 12.5 mA/Agel (duration, approx 16 hours). Resolved protein was transferred to a polyvinylidene fluoride membrane via electrotransfer at 33 V for 6.5 hours (2 gels). After transfer, the polyvinylidene fluoride membrane was blocked in 0.5% bovine serum albumin diluted in 0.1M PBS solution with 0.5% Tween 20 (P, 7.4) at 4°C for 16 to 18 hours. Following blocking, the membrane was washed in PBS-Tween solution and incubated with horseradish per-oxidase-conjugated polyclonal rabbit anti-human vWF antibody diluted 1:500 in PBS-Tween 20 solution. After a 2-hour incubation at 21°C, the membrane was washed 5 times in PBS-Tween 20 solution (5 to 10 min/wash). The VWF multimers were visualized by use of 0.05% 3,3-diaminobenzidine HCl in 0.05M Tris-HCl with hydrogen peroxide. The VWF multimer profiles were evaluated qualitatively by a person who was unaware of the source of the plasma samples with regard to the identity of the dogs and their clinical findings.

Results

The groups of dogs differed with regard to age, weight, and severity of MVP (Table 1). The CKCSs with moderate to severe MR were significantly older than CKCSs with no or minimal MR and control dogs although the CKCSs with moderate to severe MR were significantly older than those with mild MR, this difference was not significant. The mean weight of the control dogs was significantly greater than that of each of the 3 groups of CKCSs. As mentioned, none of the dogs had hematologic or serum biochemical abnormalities (except for some CKCSs that had low platelet counts).

Findings in control dogs—In the control dogs, the ranges of the measured hemostatic variables were as follows: PT, 5.9 to 7.4 seconds; APTT, 10.4 to 12.4 seconds; plasma fibrinogen concentration, 1.01 to 3.01 mg/mL; plasma D-dimer concentration, 0.14 to 0.45 mg/L; plasma vWF concentration, 64% to 167%; closure time (assessed by use of the platelet function analyzer), 43 to 83 seconds; and manual platelet count,
CKCSs had very prolonged APTTs (control dogs (range, 10.4 to 12.4 seconds). Nine values of APTT among the groups. However, a large variation of the control dogs. There was no difference in values among the 3 CKCS groups, and both sexes were represented.

### Table 1—Sex, age, weight, platelet count and function, severity of mitral valve prolapse (MVP), hemostatic variables, and plasma von Willebrand factor (vWF) concentration of 24 clinically normal dogs (control group; n = 24) and 76 untreated Cavalier King Charles Spaniels (CKCSs) with clinically inapparent mitral regurgitation (MR) as a result of myxomatous mitral valve disease.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control dogs</th>
<th>No or minimal MR (n = 44)</th>
<th>Mild MR (11)</th>
<th>Moderate to severe (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (No. of males, No. of females)</td>
<td>14, 10</td>
<td>18, 26</td>
<td>6, 5</td>
<td>14, 7</td>
</tr>
<tr>
<td>Age (mo)</td>
<td>47 (25–63)</td>
<td>25 (23–56)</td>
<td>57 (48–61)</td>
<td>68 (58–96)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>26.0 (14.3–30.0)</td>
<td>8.5 (7.6–9.4)</td>
<td>8.7 (7.3–11.5)</td>
<td>10.0 (9.1–11.9)</td>
</tr>
<tr>
<td>MVP* (mm)</td>
<td>–0.6 (–1.6–0.1)</td>
<td>1.3 (1.2–1.7)</td>
<td>2.2 (1.5–2.5)</td>
<td>§</td>
</tr>
<tr>
<td>PT (s)</td>
<td>6.6 (6.4–8.8)</td>
<td>6.3 (5.8–6.8)</td>
<td>6.4 (6.2–6.6)</td>
<td>§</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>10.4 (10.4–10.7)</td>
<td>10.4 (10.4–21.7)</td>
<td>10.4 (10.4–53.7)</td>
<td>10.7 (10.4–34.5)</td>
</tr>
<tr>
<td>Plasma fibrinogen (mg/mL)</td>
<td>1.25 (1.10–1.65)</td>
<td>2.07 (1.69–2.42)</td>
<td>1.95 (1.34–3.10)</td>
<td>2.39 (2.12–3.01)</td>
</tr>
<tr>
<td>Plasma D-dimer (µg/mL)</td>
<td>0.16 (0.14–0.21)</td>
<td>0.17 (0.15–0.20)</td>
<td>0.19 (0.13–0.32)</td>
<td>0.18 (0.16–0.19)</td>
</tr>
<tr>
<td>Plasma vWF concentration (%)</td>
<td>92 (78–126)</td>
<td>99 (75–115)</td>
<td>86 (79–94)</td>
<td>72 (53–87)</td>
</tr>
<tr>
<td>Col+ADP CT (s)</td>
<td>55 (49–65)</td>
<td>54 (51–62)</td>
<td>61 (56–81)</td>
<td>93 (85–132)</td>
</tr>
</tbody>
</table>

With the exception of sex, data are shown as medians (25th to 75th percentiles).

*Severity of MVP was assessed as the mean of the maximal protrusion (in increments of 0.5 mm) of the anterior and posterior leaflets of the mitral valve and the coaptation point of the 2 leaflets into the left atrium during systole. §Plasma D-dimer concentration was determined in only 8 control dogs. ||Value is significantly (P < 0.05) different from that of the control group. §Value is significantly (P < 0.05) different from that of the CKCSs with no or minimal MR. ||Value is significantly (P < 0.05) different from that of CKCSs with mild MR.

| PT = Prothrombin time. APTT = Activated partial thromboplastin time. Col+ADP CT = Closure time measured by use of a platelet function analyzer with collagen and adenosine diphosphate as the combined agonist. |

Hemostatic profile and plasma vWF concentration—The PIs in CKCSs with no or minimal MR and those with moderate to severe MR were shorter than that of the control dogs. There was no difference in values of APTT among the groups. However, a large variation in APTT was found among the CKCSs (range, 10.4 to 151 seconds), compared with values among the control dogs (range, 10.4 to 12.4 seconds). Nine CKCSs had very prolonged APTTs (> 60 seconds); those dogs were equally distributed among the 3 groups of CKCSs, and both sexes were represented (data not shown). There was no relationship between plasma vWF concentration and APTT in the CKCSs (data not shown). In all CKCS groups, the plasma fibrinogen concentration was significantly greater than that of the control group. There was no difference in plasma D-dimer concentration among the 4 groups. Among the 3 CKCS groups, there was no difference in any of the 4 variables included in the hemostatic profile (ie, APTT, PT, and plasma fibrinogen and D-dimer concentrations). The CKCSs with moderate to severe MR had significantly lower plasma vWF concentration, compared with that of CKCSs with no or minimal MR and control dogs (Figure 1).

**Effects of disease-related variables and other factors on plasma vWF and fibrinogen concentrations**—A significant inverse relationship was found between plasma vWF concentration (dependent variable) and each of the following variables: LVEDD, MVP severity, weight, and regurgitant jet size. When plasma fibrinogen concentration was the dependent variable, the LVEDD and LA:Ao ratio were significant disease-related predictors (Table 2; Figure 2). The relationships of age, sex, or platelet count with plasma vWF and fibrinogen concentration did not reach significance in any of the analyses. Plasma concentrations of vWF or fibrinogen in the 53 CKCSs with platelet counts > 100,000 platelets/µL did not differ from the concentrations in the 23 CKCSs with platelet counts ≤ 100,000 platelets/µL. The significant predictors generally accounted for only a minor part of the variability in plasma concentrations of vWF and fibrinogen.

**Relationship between plasma vWF concentration and closure time in the CKCSs and control dogs—**
Table 2—Assessment of the influence of disease-related and other variables on plasma vWF and fibrinogen concentrations in 76 untreated CKCSs with clinically inapparent MR as a result of myxomatous mitral valve disease by use of multiple regression analyses.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Disease-related variable assessed</th>
<th>Significant predictor*</th>
<th>$R^2$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF concentration†</td>
<td>LVEDD‡</td>
<td>LVEDD</td>
<td>0.13</td>
<td>0.001</td>
</tr>
<tr>
<td>MVP severity</td>
<td>MVP severity</td>
<td>0.17</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>0.08</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Jet size</td>
<td>Jet size</td>
<td>0.21</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>LA:Ao ratio</td>
<td>Weight</td>
<td>0.17</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen concentration§</td>
<td>LVEDD</td>
<td>LVEDD</td>
<td>0.10</td>
<td>0.005</td>
</tr>
<tr>
<td>MVP severity</td>
<td>NS</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Jet size</td>
<td>Weight</td>
<td>0.10</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>LA:Ao ratio</td>
<td>LA:Ao ratio</td>
<td>0.11</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

The $R^2$ value indicates the variable-related variance.

*In each analysis, age, sex, weight, platelet count, and a disease-related variable were included as possible predictors. Only the variables for which $P < 0.01$ are reported. All significant predictors were inversely related to plasma vWF concentration. Normalized to dog size (value divided by weight [kg]$^{1/29}$). All significant predictors were directly related to plasma fibrinogen concentration.

LVEDD = Left ventricular end-diastolic diameter. LA:Ao ratio = Left atrial-to-aortic root ratio. NS = Not significant. NA = Not applicable.

See Table 1 for remainder of key.

![Figure 2](image-url)

Figure 2—Relationships between echocardiographic variables and plasma vWF and fibrinogen concentrations in 76 untreated CKCSs with different degrees of clinically inapparent MR. A—Scatterplot of left ventricular end-diastolic diameter (LVEDD) versus plasma vWF concentration ($R^2 = 0.13; P = 0.001$). B—Scatterplot of the severity of mitral valve prolapse versus plasma vWF concentration ($R^2 = 0.17; P < 0.001$). C—Scatterplot of LVEDD versus plasma fibrinogen concentration ($R^2 = 0.10; P = 0.005$). D—Scatterplot of left atrial-to-aortic (LA:Ao) ratio versus plasma fibrinogen concentration ($R^2 = 0.11; P = 0.003$). Each value of LVEDD was divided by weight (kg)$^{1/29}$ to normalize it to the size of the dog.
the CKCSs, a significant inverse relationship was identified between plasma vWF concentration and closure time \( (R^2 = 0.15; P < 0.001) \), with regurgitant jet size as a covariate \( (R^2 = 0.53; P < 0.001) \); Figure 3). No significant interaction was detected between plasma vWF concentration and regurgitant jet size. Closure time was significantly longer in the CKCSs with moderate to severe MR, compared with closure times in CKCSs with no or minimal MR, CKCSs with mild MR, and control dogs. Closure time was significantly longer in CKCSs with mild MR, compared with closure time in CKCSs with no or minimal MR (Table 1). In the control dogs, no relationship between closure time and either plasma vWF concentration or regurgitant jet size was identified \( (P = 0.97 \text{ and } 0.88, \text{ respectively}) \).

Multimeric composition of vWF in CKCSs, compared with that of control dogs—Western blot analyses revealed a relative absence of high molecular weight bands in the samples obtained from CKCSs with moderate to severe MR (ie, CKCSs with regurgitant jet size of 55% to 90%) and a vWF concentration < 54%, compared with samples obtained from CKCSs with no or minimal MR and control dogs with a vWF concentration of 78% to 132% or 54% to 69%. This finding indicated that CKCSs with moderate to severe MR had less high-molecular-weight multimers (HMWMs) than the other study dogs (Figure 4).

**Discussion**

The results of the study reported here indicated that all CKCSs with MVP had higher plasma fibrinogen concentration than that of clinically normal control dogs, and a weak direct association was identified between the plasma fibrinogen concentration and 2 disease-related variables (LVEDD and LA:Ao ratio) in the CKCSs. Furthermore, the plasma vWF concentration was lower in CKCSs with moderate to severe MR, compared with the concentrations in the CKCSs with no or minimal MR and the control dogs. Qualitative analysis of the multimeric composition of vWF indicated less HMWMs in CKCSs with MR and a plasma vWF concentration of 45% to 54%, compared with the amount in CKCSs with no MR and a plasma vWF concentration of 101% to 127% (the amount in control dogs with a plasma vWF concentration of 78% to 132%) and the amount in control dogs with a plasma vWF concentration of 54% to 69%.

In humans with cardiovascular disease, high plasma fibrinogen concentration is associated with an increased risk
of thrombosis and is an independent marker of cardiovascular risk. Fibrinogen is also an acute phase protein; therefore, its concentration in plasma may vary greatly. Despite the variability in plasma concentration, the association between plasma fibrinogen concentration and cardiovascular disease in humans based on a single measurement is strong and consistent. An increase in plasma fibrinogen concentration has been reported in humans with MR, compared with the concentration in unaffected individuals. In our study, we detected higher plasma fibrinogen concentration in CKCSs with MR, compared with that of control dogs; furthermore, there was a relationship between plasma fibrinogen concentration and both the LVEDD and LA:Ao ratio in the CKCSs, indicating that the changes in plasma fibrinogen concentration might be associated with the mitral valve disease. However, large epidemiologic studies to investigate plasma fibrinogen concentration and cardiovascular risk in dogs, as well as breed variability studies, are needed before any broader conclusions regarding plasma fibrinogen concentration in dogs with cardiovascular disease can be made.

Measurement of plasma D-dimer concentration has been used to detect thromboembolic disease in dogs. It appears that plasma D-dimer concentration has a high negative predictive value for thromboembolic disease in dogs as it does in humans and that only very high plasma concentrations of D-dimer have a high positive predictive value. In the present study, we did not detect any differences in plasma D-dimer concentration among the groups of dogs. Consequently, it might be concluded that there is no active thromboembolic disease in dogs with MR or that the thromboembolic disease is too mild to cause a noticeable change in plasma D-dimer concentration in those dogs.

Assessment of APTT and PT were included in the hemostatic profile in our study because of the limited availability of assays suitable for measuring markers of hypercoagulability in dogs. Thrombogenesis is a complex process involving platelets, leukocytes, endothelial cells, and fluid-phase hemostatic proteins. Because of this complexity, it has been suggested that in vitro coagulation screening tests designed to detect factor deficiencies (ie, assessment of APTT and PT) are not good predictors of an in vivo state of hypercoagulation. However, in humans, a shortened APTT is associated with increased risk of acute myocardial infarction. No significant difference in values of APTT was found among the groups of dogs in our study, although the APTT varied tremendously among the CKCSs. A hereditary single factor deficiency (without clinical evidence of bleeding) could be speculated to cause severe prolongation of APTT. Single factor analyses in CKCSs with prolonged APTTs would indicate whether a hereditary single coagulation factor deficiency is present in those dogs. However, other causes of prolonged APTT such as acquired coagulopathies cannot be ruled out, although this is considered unlikely because the dogs in our study had no signs of clinical disease. The clinical importance of the shorter PT detected in some CKCSs in our study, compared with PTs in the control dogs, is unknown. The finding was not consistent in all the groups of CKCSs and was not associated with any disease-related variable.

An interesting result of the study reported here was that CKCSs with moderate to severe MR had a lower plasma vWF concentration, compared with the concentrations in CKCSs with no or mild MR and control dogs. The wide overlap between the 4 groups of dogs was likely a result of the large variation in plasma vWF concentration and the fact that regurgitant jet size can be regarded only as a semiquantitative measure of the degree of MR. However, the significant relationship between plasma vWF concentration and 2 other disease-related variables (LVEDD and MVP) supports the finding of a difference in plasma vWF concentration among the groups. In our study, a strong association between plasma vWF concentration and the closure time measured by use of the platelet function analyzer was identified in CKCSs. Because of the wide range of plasma vWF concentration (64% to 167%) found in control dogs in this study, the same association might be expected in the control dogs; however, no relationship was identified between plasma vWF concentration and closure time in the control dogs. The reason for this discrepancy could be that CKCSs with MR, low plasma vWF concentrations, and long closure times also have a loss of the HMWMs of vWF and that a loss of HMWMs has a greater influence on closure time than does total plasma vWF concentration. This could explain the lack of correlation between plasma vWF concentration and closure time in the control dogs.

In humans, a high plasma vWF concentration has been a consistent finding in patients with cardiovascular disease, including individuals in whom atherosclerosis is thought not to be involved in the pathophysiologic development of the cardiovascular disease (such as patients with idiopathic dilated cardiomyopathy, severe mitral regurgitation, and aortic valve disease). Furthermore, plasma vWF concentration is regarded as an excellent marker of endothelial dysfunction in humans. However, decreased plasma vWF concentration has been detected in some patients with MVP and congenital cardiac defects, but the methods used for measuring plasma vWF concentration in those earlier studies may have been different from the methods used in this study.
Methods used at present. Therefore, we hypothesized that CKCSs with MR in our study would have a greater plasma vWF concentration, compared with the concentration in control dogs; however, a lower plasma vWF concentration was detected in CKCSs with MR. It is plausible that some form of endothelial dysfunction is present in dogs with heart disease. First, results of a study\(^\text{12}\) in dogs indicated that metabolites of nitric oxide were present in lower amounts in CKCSs with mild, moderate, or severe MR, compared with the amounts in CKCSs with no MR and control dogs, suggesting that MR-affected dogs have endothelial dysfunction. Second, other investigations have revealed that coronary endothelial dysfunction precedes experimentally induced heart failure in dogs\(^\text{18}\) and is present after episodes of ischemia and reperfusion in dogs.\(^\text{39}\) Finally, endothelial dysfunction is generally associated with all forms of heart failure in humans. Therefore, it can be speculated that plasma vWF concentration might not be a good marker of endothelial dysfunction in dogs with diseases that involve substantial shear stress to the blood. Further studies are warranted to elucidate whether dogs are generally different from humans with regard to the usefulness of plasma vWF concentration as a marker of endothelial dysfunction.

Results of the evaluation of the multimeric composition of vWF in the study dogs indicated a loss of HMWMs in the 4 CKCSs with MR and low plasma vWF concentration, compared with the other CKCSs and control dogs evaluated. In humans, low plasma vWF concentration and bleeding tendency have been associated with MVP\(^\text{34-36}\) and a decrease in the HMWMs of vWF has been reported in patients with aortic stenosis\(^\text{30,31}\) and congenital cardiac defects.\(^\text{37}\) It has been speculated that the decrease in HMWMs is caused by accelerated clearance of the largest multimers via accelerated platelet and vWF interactions in blood flowing through a stenotic aortic or regurgitant mitral valve.\(^\text{38}\) However, results of studies\(^\text{39-41}\) have indicated high shear stress per se can alter the molecular conformation of vWF, thereby leading to an enhanced proteolytic susceptibility of vWF molecules that may account for the loss of HMWMs. We speculate that CKCSs with MR have a decrease in plasma vWF concentration and HMWMs (compared with unaffected dogs) as a result of increased turbulence and shear stress to the blood. A weakness of our study is that vWF multimer analysis was performed in only 16 selected dogs (ie, 8 control dogs and 8 CKCSs). Therefore, it is not possible to conclude whether there is a loss of HMWMs of vWF in CKCSs with MR and normal vWF concentration and whether CKCSs without MR but with low plasma vWF concentration also have a loss of HMWMs. However, if CKCSs without MR and with low plasma vWF concentration have a loss of HMWMs, long closure times (measured by the platelet function analyzer)—not normal times such as those we identified in those dogs—would be expected. In addition, because all of the 4 CKCSs with MR and low plasma vWF concentration but none of the control dogs with low plasma vWF concentration included in the vWF multimer analysis had a loss of HMWMs, these results suggest that there is a loss of HMWMs in CKCSs with MR, which results in low plasma vWF concentration.

Overall, our data indicated that the fibrinogen concentration in plasma was higher in all CKCSs, compared with that of control dogs, and was associated with values of the LVEDD and LA:Ao ratio in CKCSs. Surprisingly, a lower plasma vWF concentration was detected in CKCSs with moderate to severe MR, compared with the concentrations in the CKCSs with no or minimal MR and control dogs. The explanation for this finding is a matter of speculation, but it is suggested that there is increased destruction of the HMWMs of vWF via shear stress in the CKCSs with more severe MR, thereby reducing the total amount of plasma vWF (as indicated by the results of the quantitative ELISA). Because of this phenomenon, plasma vWF concentration may not be useful as a marker of endothelial dysfunction in canine diseases that involve considerable shear stress to the blood. The clinical importance of the changes in the hemostatic markers that were evaluated in our study, their role in the pathophysiologic progression of MMVD, and the risk of thromboembolism in dogs with MR remain to be elucidated.

References