Brief Communication
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Von Willebrand Factor Antigen Concentration in Dogs with Sepsis

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Background: Von Willebrand factor (vWF) antigen concentration, a marker of endothelial activation, is increased in human patients with multiorgan failure, sepsis, or both, and is an independent predictor of survival.

Hypothesis/Objectives: vWF antigen concentrations are significantly higher in dogs with sepsis.

Animals: Fourteen dogs hospitalized with sepsis. Sepsis was defined as microbiologic or cytologic evidence of infection combined with systemic inflammatory response syndrome. Control dogs were healthy dogs, without evidence of disease.

Methods: Prospective, observational study. Dogs admitted to the intensive care unit with a diagnosis of sepsis were considered eligible for enrollment into the study. Exclusion criteria included a previous diagnosis of von Willebrand disease or a recent history of a plasma transfusion. Citrated plasma samples were collected for analysis of vWF antigen by ELISA. All samples were drawn from dogs during hospitalization. Data between populations were analyzed using nonparametric statistical analysis with a P value $< .05$ considered significant.

Results: Twenty-five dogs were enrolled; 14 dogs with sepsis and 11 control dogs. The median vWF antigen concentration in dogs with sepsis was 156% (range, 117–200%), which was significantly higher than healthy dogs (105%; range, 44–155%, $P < .005$). There was no difference between survivors and nonsurvivors with a median vWF antigen concentration of 144% (range, 136–201%) in survivors (n = 7) and 159% (range, 122–174%) in nonsurvivors (n = 7) ($P = .5$).

Conclusions and Clinical Importance: vWF is increased in dogs with sepsis, possibly reflecting endothelial activation. Further exploration of endothelial function is warranted in critically ill dogs.

Key words: Biomarkers; Critical care; Endothelial injury; Inflammation.

Endothelial injury and activation leads to the release of von Willebrand Factor (vWF) from preformed stores in endothelial cells and increased concentrations of plasma vWF have been associated with a variety of diseases in people, including pulmonary hypertension, nephritis, diabetes mellitus, ischemic heart disease (myocardial infarction), chronic renal failure, rheumatologic inflammatory diseases, and severe infection.[1–3] Plasma vWF concentrations are significantly increased in human patients with multiorgan failure or sepsis compared with other populations[1,3–5] and might serve as an independent predictor of survival in certain populations.[6] vWF is a particularly appealing marker for evaluation of endothelial activation in veterinary medicine as it can be measured in practice. The goal of this study was to evaluate vWF activity in dogs with sepsis, a known disease of critical illness with the hypothesis that increases in vWF will be seen in dogs, as in people.

Materials and Methods

Dogs admitted to the intensive care unit with a diagnosis of sepsis were eligible for enrollment into the study. The diagnosis of sepsis was defined as cytopathologic or microbiologic confirmation of infection and meeting 2 of the following criteria: hypothermia or hyperthermia ($<100^{\circ}$F, $>103^{\circ}$F), tachycardia (>140 beats/min), tachypnea (>20 breaths/min), and leukopenia ($<6{,}000$ cells/μL), leukocytosis ($>16{,}000$ cells/μL), or $>3\%$ band neutrophils.[7] Aerobic or anaerobic bacterial cultures were performed for all dogs. Blood cultures were performed for 2/14 dogs. The study protocol was approved by the Tufts University Institutional Animal Care and Use Committee. A control population of healthy dogs belonging to the students and staff of the Tufts Cummings School of Veterinary Medicine was also enrolled. All blood samples were collected either via venipuncture or from previously placed central venous catheters into citrated tubes (1 part 3.8% citrate: 9 parts whole blood). Blood was centrifuged within 30 minutes of collection, separated and citrated plasma was stored at -40°C until batch analyzed for vWF antigen concentration at Cornell Comparative Coagulation Laboratory (Coagulation Section-Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University). Guidelines for appropriate submission were met and the samples were analyzed by an ELISA.[8] All dogs that were known to have received a plasma transfusion within 7 days were excluded, as were dogs with a known or a suspected history of inherited von Willebrand disease. Twenty-five dogs were enrolled into the study: 14 dogs with sepsis and 11 healthy control dogs. Dogs were divided into survivors and nonsurvivors. Von Willebrand antigen concentrations were compared between the study population and controls, as well as between survivors and nonsurvivors. The data were analyzed using nonparametric statistics. A 2-tailed Mann-Whitney test was used to compare septic versus control dogs as well as used to compare survivors to nonsurvivors. Results were considered significant at $P < .05$. Commercial statistical software was used.[a,b]

Results

The most common source of sepsis was abdominal (n = 7), followed by thoracic (n = 5), musculoskeletal (n = 1), and bacteremia (n = 1). Causes of abdominal sepsis included foreign body with intestinal perforation (n = 5), urinary tract infection (n = 1), and gall bladder mucocele (n = 1). Thoracic sources of sepsis included pyothorax (n = 2), pulmonary abscess (n = 1), bacterial pneumonia (n = 1), and fungal pneumonia (n = 1). One dog had positive blood cultures with signs of sepsis, without an identified underlying focus. Seven dogs survived to discharge and 7 died or were euthanized. The median vWF antigen concentration in dogs with sepsis was 156%

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(range, 117–200%), which was significantly higher than in control dogs (105%; range, 44–155%, $P < .005$). There was no statistical difference between survivors and nonsurvivors. The median vWF concentration was 144% (range, 136–201%) in survivors (n = 7) and 159% (range, 122–174%) in nonsurvivors (n = 7) ($P = .5$).

Discussion

We found that vWF antigen concentration is significantly higher in dogs with sepsis and we propose that vWF might serve as a marker for endothelial activation in critically ill patients.

Many diseases of critical illness, including pancreatitis, hypoxia, neoplasia, immune-mediated diseases, and sepsis, result in endothelial damage in people.[1,4] This insult to the endothelium is thought to be at least partially responsible for the clinical signs associated with the systemic inflammatory response syndrome described extensively in critically ill patients. Because the endothelium is a diffuse and disseminated structure, it has traditionally been difficult to access thus hampering recognition of its importance. There has been advancements in evaluating individual biomarkers in veterinary medicine with one of the most well studied being endothelin-1 in dogs with cardiac disease.[9] The primary advantage of circulating biomarkers is their clinical simplicity and the ability to quantify different factors.

vWF is increased in critically ill humans to a much greater magnitude than seen in this study of dogs. The reason for this variation is not known, but it is possible that as vWF has different cellular localizations in humans compared with dogs, the degree of increase between populations cannot be compared.[10] It is unknown to what extent the different pools contribute to the increase in vWF in critical illness and additional studies are warranted. In addition, it is possible that there are significant differences in the severity of illness between our study population and the prior studies evaluating vWF antigen concentrations in humans with sepsis.

There were several limitations to this study. A larger sample size would have allowed further stratification of cases by severity of illness as well as increased the statistical power of the study. Low statistical power means that results of this study should not be construed as indicating a lack of utility in measuring vWF antigen in predicting likelihood of death in dogs with sepsis. In addition, concurrent evaluation of other biomarkers along with functional studies would have provided a superior assessment of endothelial activation during sepsis. Finally, it has recently been demonstrated that the ratio of vWF antigen and level and activity of ADAMTS13 (vWF cleaving protease) might be more beneficial than evaluating either biomarker alone when evaluating humans with sepsis.[11] It is likely that the etiology behind increased concentrations of vWF in critical illness is multifactorial with decreased degradation (because of alterations in the activity of ADAMTS13) as well as increased production of vWF both contributing to the increases noted in both human and canine populations.

This study illustrates that an increase in vWF might provide supporting evidence for endothelial activation in critically ill dogs. With further knowledge and understanding of endothelial dysfunction, we will be better able to predict the onset and severity of systemic inflammation with the aim of modifying and targeting our therapeutics to minimize organ damage and secondary organ failure in diseases associated with critical illness.

Footnotes

[a] MS-Excel, MS Office 2003, Seattle, WA
[b] http://faculty.vassar.edu/lowry/utest.html

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