Identification of hypercoagulability in dogs with primary immune-mediated hemolytic anemia by means of thromboelastography

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Objective—To evaluate whole blood hemostasis by means of thromboelastography in dogs with primary immune-mediated hemolytic anemia (IMHA) to determine whether these dogs had evidence of hypercoagulability prior to the administration of immunosuppressant medications, blood transfusion products, or anticoagulant agents.

Design—Evaluation study.

Animals—11 client-owned dogs admitted to a teaching hospital for management of primary IMHA and 20 clinically normal dogs.

Procedures—Citrated whole blood samples were obtained from all dogs for performance of kaolin-activated thromboelastography. Citrated plasma was harvested from blood samples of dogs with IMHA for plasma-based coagulation testing, including activated partial thromboplastin time, prothrombin time, D-dimer concentration, fibrinogen concentration, and antithrombin activity.

Results—Compared with control dogs, dogs with primary IMHA had evidence of hypercoagulability as indicated by a significantly lower median (range) clot formation time (0.8 seconds [0.8 to 2.0 seconds] vs 1.9 seconds [1.3 to 3.8 seconds]), higher median angle (76.3° [66.3° to 84.6°] vs 64.0° [45.4° to 71.0°]), higher median maximum amplitude (75.9 mm [59.2 mm to 86.3 mm] vs 55.7 mm [49.9 mm to 63.6 mm]), and higher median clot strength (15,000 dyne/cm² [9,900 to 31,400 dyne/cm²] vs 6,100 dyne/cm² [4,900 to 8,700 dyne/cm²]).

Conclusions and Clinical Relevance—Dogs with primary IMHA had hypercoagulability as demonstrated by thromboelastography at the time of initial diagnosis and prior to treatment. Such hypercoagulability may be a precursor to clinically evident thrombosis as a complication of the disease process. (J Am Vet Med Assoc 2011;238:463–467)

Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>α</td>
<td>Angle</td>
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<td>aPTT</td>
<td>Activated partial thromboplastin time</td>
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<td>AT</td>
<td>Antithrombin</td>
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<td>G</td>
<td>Clot strength</td>
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<td>IMHA</td>
<td>Immune-mediated hemolytic anemia</td>
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<td>K</td>
<td>Clot formation time</td>
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<td>MA</td>
<td>Maximum amplitude</td>
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<td>PT</td>
<td>Prothrombin time</td>
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<td>R</td>
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Immune-mediated hemolytic anemia is a condition in which autoantibodies bind to the erythrocyte membrane and lead to the premature destruction of RBCs. This condition is the most common hemolytic disorder in dogs, and it can be classified as a primary or secondary disease process. Various causes have been associated with the onset of secondary IMHA including neoplasia, infectious diseases, and drug or toxicant exposure. However, in most situations, a cause or coincidental condition other than breed predilection is not identified, and IMHA is categorized as a primary autoimmune disorder. Primary canine IMHA is more common than secondary IMHA.

In dogs, IMHA has a variable survival rate, with mortality rates reported as high as 70%.[1,2] Thromboembolism has been cited as the most common complication in dogs with primary IMHA, and its prevalence as determined at necropsy is reportedly as high as 80%.[3,4] Despite strong suspicion for the role of hypercoagulability in the clinical signs and death of dogs with primary IMHA, antemortem determination of such a state in association with the disease process alone has not been reported to the authors’ knowledge. The association of thromboembolism with various treatment strategies including immunosuppressant medication, IV catheterization, and blood product transfusion further complicates the clinical scenario. Confirmation of a hypercoagulable state prior to the development of overt thrombosis, and in association with the disease process alone, would provide support for the integration of early anticoagulant treatment. However, a method by which to reliably identify hypercoagulability prior to treatment has not yet been reported.

Conventional coagulation parameters including PT and aPTT help identify hypocoagulable states but are considerably less helpful in the detection of hypercoagulability.[5,6] A high plasma fibrinogen concentration, low blood platelet count, and low plasma AT activity can be

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associated with thrombosis; however, these findings are not consistent or reliable predictors of hypercoagulability.9–10 A high plasma D-dimer and fibrin degradation products concentration are suggestive of thrombosis and fibrinolysis, but these can be high in various disorders and are also not considered reliable indicators of hypercoagulability.9–10 Prothrombin time, aPTT, fibrinogen concentration, AT activity, and D-dimer concentration have a high degree of individual variation in dogs, making population-based reference limits an insensitive interpretation criterion.11

Thromboelastography is an analytic in vitro point-of-care test that provides a thorough assessment of hemostatic function in whole blood with evaluation of both plasma and cellular components of hemostasis.9–10 The technique measures the viscoelastic changes that occur during the interaction between fibrinogen, platelets, and the protein coagulation cascade. It allows evaluation of the entire coagulation system from clot initiation and clot formation through fibrinolysis. Thromboelastography measures all dynamic aspects of coagulation and has been used to detect hypercoagulability in humans and other animals.9–10 Characteristic changes in thromboelastography variables are typically observed when hypercoagulable blood is tested, producing a tracing that reflects alterations in platelet function and activation of coagulation proteases, inhibitors, and the fibrinolytic system. Thromboelastography can detect hemostatic aberrations in the absence of changes in the coagulation parameters traditionally evaluated and permits an assessment of thrombotic risk when more conventional coagulation tests fail to do so.9–10 Thromboelastography parameters have a low degree of variation among healthy dogs, allowing conventional population-based reference limits to be a sensitive interpretation criterion.11

We hypothesized that dogs with primary IMHA are hypercoagulable at the time of diagnosis and prior to administration of immunosuppressant agents, anticoagulants, or blood product transfusions. We further hypothesized that because of the difficulties in identifying and documenting a hypercoagulable state by using routine plasma-based coagulation screening tests, hypercoagulability in dogs with primary IMHA could be evaluated through whole blood hemostasis by using kaolin-activated thromboelastography at admission to the teaching hospital and prior to administration of immunosuppressant medications, blood transfusion products, or anticoagulant agents.

Materials and Methods

Animals—Dogs were enrolled during a 1-year period from 2008 to 2009. Client-owned dogs admitted to the teaching hospital of the Cummings School of Veterinary Medicine at Tufts University, with a new diagnosis of primary IMHA were eligible for inclusion, provided they had not received anticoagulant or immunosuppressive treatment or blood products. A diagnosis of IMHA was made on the basis of the presence of anemia (Hct < 26%) and evidence of either or both autoagglutination and spherocytosis as confirmed by a board-certified clinical pathologist. All dogs had a complete history, physical examination, CBC, and serum biochemical analysis and underwent survey thoracic radiography and abdominal ultrasonography. All imaging results were reviewed by a board-certified radiologist. Secondary IMHA was ruled out when no cause was identified on the basis of historical information or findings of physical examination, laboratory work, or diagnostic imaging. Clinically normal dogs owned by hospital staff and veterinary students served as the control group for thromboelastography. These dogs were determined healthy on the basis of their history and results of physical examination, CBC, and serum biochemical analysis. The study protocol was approved by the Cummings School of Veterinary Medicine at Tufts University Clinical Studies Review Committee, and written consent was obtained from all owners.

Blood sample collection—All blood samples from dogs with IMHA were obtained upon admission to the hospital and prior to any treatment. Blood samples were also obtained from control dogs. Each sample was collected via cephalic, jugular, or saphenous venipuncture with minimum stasis and a 21-gauge butterfly needle into 2 citrated plastic plasma tubes. The 2.7-mL citrate tubes were inverted gently to ensure mixing of 3.2% trisodium citrate and blood in a 1:9 ratio. One tube was stored at room temperature (approx 20°C) for 30 minutes prior to thromboelastography. The remaining tube was centrifuged immediately to obtain citrated plasma, which was subsequently stored at −80°C until batch coagulation profile analysis was performed.

Thromboelastography—Thromboelastography was performed in the Clinical Sciences Laboratory at the Cummings School of Veterinary Medicine at Tufts University with an automated hemostasis analyzer.5 For each sample, 20 µL of calcium chloride was placed in a prewarmed thromboelastography cup. One milliliter of citrated blood was added to a vial of standardized kaolin130 activator and mixed in accordance with the manufacturer’s directions. Once mixed, 340 µL of the kaolin-activated sample was added to the thromboelastography cup for a total volume of 360 µL in each cup, and the analysis was performed. Analyses were run at 37°C until the MA was reached. Variables recorded included R, K, α, and MA. The R represents the interval from the initiation of analysis until the initial detection of clot formation; it is related to plasma clotting factors and inhibitor activity. The K represents the interval to clot formation, measured from the end of the R until the amplitude of the tracing reaches 20 mm; it is related to clotting factors, fibrinogen, and platelets. The α is the slope of the thromboelastogram, which represents the rate of clot formation and the rapidity of fibrin buildup and cross-linking; it is mainly dependent on the concentrations of platelets, fibrinogen, and clotting factors. The MA is the widest point of the tracing and is a direct function of fibrin and platelet bonding. The MA is used to derive clot shear elastic modulus or global G, whereby G = 5,000 × MA/100 – MA). Values consistent with hypercoagulability include a reduced R, reduced K, increased α, increased MA, and increased G when compared with reference values.9–10

Coagulation testing—Activated partial thromboplastin time, PT, AT activity, fibrinogen concentration, and D-dimer assays were performed on batched citrated plasma samples by the Cornell Animal Health Diagnostic Center. The aPTT, PT, and fibrinogen determinations were performed by use of an automated mechanical clot detection instrument.5 The aPTT reagent14 was used with an activation time of 180 seconds. The PT assay was performed by use of a rabbit brain phospholipid reagent. The fibrinogen assay was performed via the Clauss method by use of a human thrombin reagent,5 and a standard curve was derived
from dilutions of a canine plasma standard. The fibrinogen concentration of the canine plasma standard is determined by a quantitative, multispecies fibrinogen ELISA. Plasma AT activity was measured with a synthetic chromogenic substrate kit in accordance with the manufacturer's recommendations for assay method and instrumentation, with minor modifications in sample dilution and substitution of pooled plasma from hematologically normal dogs, rather than human plasma, for the calibration standard. Activities of plasma AT are reported as the percentage of the canine standard, which had an assigned value of 100% activity. Plasma D-dimer concentration was measured in a quantitative, turbidimetric immunoassay with a human D-dimer calibration standard, in accordance with the manufacturer's recommendations for assay method.

Statistical analysis—Distributions of data were examined graphically for normality. Statistical analysis was performed with the Mann-Whitney U test to compare thromboelastography values from dogs with IMHA with those from control dogs. Significance was set at a value of P < 0.05, and a statistical software package was used to analyze the data. Descriptive statistics were used to summarize dog characteristics at hospital admission as well as findings of CBCs, serum biochemical analyses, and coagulation testing for dogs with IMHA. Results are expressed as median (range) unless indicated otherwise.

Results

Animals—Twenty clinically normal dogs were enrolled as the control group for performance of thromboelastography. Eighteen dogs were identified as potentially eligible for inclusion in the IMHA group. Four of these dogs were later excluded because they were discharged or euthanatized prior to having all necessary screening tests, 2 dogs were excluded because of a lack of confirmation of autoagglutination or spherocytosis from the clinical pathology laboratory, and 1 dog was excluded because it had intra-abdominal neoplasia. The remaining 11 dogs with IMHA consisted of 8 spayed females and 3 castrated males. There were 2 Cocker Spaniels, 2 English Springer Spaniels, and 1 of each of the following breeds: Maltese, Golden Retriever, Miniature Pinscher, Standard Poodle, Chesapeake Bay Retriever, Labrador Retriever, and mixed breed. The median (range) age of dogs with IMHA was 6 years (0.67 to 11 years).

The median Hct of dogs with IMHA at admission was 17% (range, 10% to 20%; reference limits, 39% to 55%). Autoagglutination and spherocytosis were present in all 11 dogs. The median rectal temperature was 39.1°C (102.4°F; 38.6° to 40.2°C [101.4° to 104.3°F]), and the median heart rate was 140 beats/min (120 to 190 beats/min). The median body weight was 19.9 kg (43.8 lb; 3 to 38.9 kg [6.6 to 85.6 lb]). Median values of clinicopathologic variables were as follows: WBC count, 23,400 cells/µL (11,200 to 53,900 cells/µL); reference limits, 4,900 to 16,900 cells/µL; band neutrophil count, 559 cells/µL (0 to 2,823 cells/µL); reference limits, 0 to 300 cells/µL; median platelet count, 1,493,000 platelets/µL (623,000 to 1,922,000 platelets/µL); reference limits, 181,000 to 525,000 platelets/µL; median percentage of reticulocytes, 12.6% (4.6% to 24.6%; reference limits, 0% to 2.0%); serum albumin concentration, 3.4 g/dL (2.7 to 4.6 g/dL; reference limits, 2.8 to 4.0 g/dL); serum alkaline phosphatase concentration, 172 U/L (82 to 488 U/L; reference limits, 12 to 121 U/L); BUN concentration, 18 mg/dL (10 to 28 mg/dL; reference limits, 8 to 29 mg/dL); and serum total bilirubin concentration, 1.0 mg/dL (0.4 to 15.0 mg/dL; reference limits, 0.1 to 0.3 mg/dL).

The median value for aPTT was 16.5 seconds (10 to 25 seconds; reference limits, 10 to 17 seconds), and for PT was 14.0 seconds (11.0 to 19.0 seconds; reference limits, 11 to 16 seconds). All 11 dogs had a high plasma fibrinogen concentration, with a median value of 1,355 mg/dL (744 to 2,008 mg/dL; reference limits, 147 to 479 mg/dL). Ten of 11 dogs had a high plasma D-dimer concentration, with a median value of 751 ng/mL (227 to 3,164 ng/mL; reference limit, < 250 ng/mL). Three of 11 dogs had low AT activity, with a median activity of 74% (46% to 127%; reference limits, 63% to 145%).

Thromboelastography—Compared with control dogs, dogs with primary IMHA had evidence of hypercoagulability as indicated by a lower median (range) K (0.8 seconds [0.8 to 2.0 seconds] vs 1.9 seconds [1.3 to 3.8 seconds]; P < 0.001), higher median α (76.1° [59.2° to 84.6°] vs 64.8° [45.4° to 71.0°]; P = 0.001), higher median MA (75.9 mm [66.3 to 86.3 mm] vs 55.7 mm [49.9 to 63.6 mm]; P < 0.001), and higher G (15,000 dyne/cm² [9,900 to 31,400 dyne/cm²] vs 6,100 dyne/cm² [4,900 to 8,700 dyne/cm²]; P < 0.001). The R values were not significantly different between groups (P = 0.832). The thromboelastography tracings of all 11 dogs with IMHA were hypercoagulable when compared with control dogs.

Discussion

The mechanism of hypercoagulability and the cause of thromboembolism in dogs with IMHA have yet to be determined. Potential factors that have been proposed include release of procoagulant thromboplastin from the membranes of lysed RBCs, alterations in coagulation factors, platelet activation, hypoxia, release of inflammatory mediators, endothelial injury, and altered blood viscosity. Treatment with corticosteroids, the mainstay of immunosuppressant therapy, has also been suggested as a potential cause of hypercoagulability in dogs with IMHA. Historically, the presence of hypercoagulability has been difficult to establish prior to the development of overt thrombosis, principally because results of traditional plasma-based coagulation tests are unable to confirm a hypercoagulable state in affected dogs.

Changes in hemostatic parameters in dogs with IMHA have been reported, including abnormal clotting times (aPTT and PT), high D-dimer concentration, low AT activity, and high fibrinogen concentration. However, these abnormalities are neither specific nor sensitive for detecting hypercoagulability. Our study revealed that the median PT and aPTT in dogs with IMHA were not prolonged when compared with reference values. Although helpful for identifying hypercoagulability, changes in PT and aPTT cannot be used to identify hypercoagulability.

All 11 dogs with IMHA in the present study had a plasma fibrinogen concentration that exceeded the upper reference limit. Fibrinogen is an acute-phase inflammatory protein produced by the liver, and the plasma concentration of fibrinogen is high when systemic inflammation is present. However, during the formation of thrombi, fibrinogen is cleaved into fibrin monomers by thrombin, at which point...
coagulation is activated. Thus, we would expect the concentration of fibrinogen to ultimately decrease as thrombi are formed and fibrinogen stores are depleted. In our study, fibrinogen concentration was measured when dogs were initially evaluated at the hospital. Additional studies are needed to determine whether fibrinogen concentration decreases throughout the course of IMHA as a result of consumption.

Ten of 11 study dogs also had a high D-dimer concentration. D-dimers are formed when plasmin degrades cross-linked fibrin, and a high D-dimer concentration suggests ongoing active coagulation because thrombin is required for the formation of cross-linked fibrin and plasmin is required for its degradation. Thus, a high D-dimer value indicates the activation of coagulation as well as subsequent fibrinolysis. Additionally, although sensitive for detecting thromboembolic disease, an increase in D-dimer concentration is not specific for detecting thrombosis but, rather, can exist in various disease conditions.

A retrospective study in which thromboelastography was evaluated in dogs with IMHA by use of citrated whole blood samples revealed that 76% of all thromboelastography variables were consistent with a hypercoagulable state. However, in that study, all dogs had received at least 1 dose of corticosteroids and various other treatments before thromboelastography was performed. Also, the analyses were performed at varying times throughout hospitalization. Such limitations prevent the drawing of conclusions regarding the hemostatic state of dogs with IMHA at time of initial diagnosis and prior to treatment.

To the authors’ knowledge, the present study represents the first prospective evaluation of whole blood hemostasis in dogs with IMHA involving kaolin-activated thromboelastography. All dogs in the IMHA group, when compared with control dogs, had evidence of hypercoagulability as indicated by significantly shortened K, high α, high MA, and high G values. Given these findings, hypercoagulability is likely present in dogs with primary IMHA upon initial diagnosis and prior to any treatment. The hypercoagulable state identified via thromboelastography may be a precursor to clinically evident thrombosis, and results suggest that institution of anticoagulation treatment may be warranted early in the course of the disease.

In a recent study, tissue factor–activated thromboelastography was used to demonstrate the development of hypercoagulability in 6 healthy Beagles treated with prednisone at a dosage of 1 mg/kg (0.5 mg/lb) daily for 2 weeks. Our study showed that the hypercoagulable state in dogs with primary IMHA preceded corticosteroid treatment. Therefore, it is possible that once treatment is initiated, corticosteroid administration may worsen or contribute to the preexisting hypercoagulable state and risk for thromboembolism.

A limitation of the present study is the use of healthy dogs as a control group. Anemia alone may alter blood viscosity secondary to a decrease in RBC mass and relative increase in plasma proteins concentration. In another study, alterations in thromboelastographic values were detected when whole blood from healthy dogs was serially diluted with aliquots of autologous plasma to alter the Hct value, creating anemia with a platelet count within reference limits. That study revealed that the MA increases with decreasing Hct. However, it is unknown whether this finding is an in vitro artifact associated with a relative deficit of sodium citrate per volume of plasma or an in vivo phenomenon, with the RBC mass itself truly altering hemostasis. Because the mechanism of alterations in thromboelastography values remains uncertain, the use of anemic control dogs with similar Hct values due to a cause other than IMHA or adjustment of the amount of sodium citrate added to the whole blood sample from an anemic dog in future studies of IMHA may help to answer this question. However, the ideal control population with spontaneously occurring anemia, without concurrent coagulation abnormalities or risk for thromboembolism, remains unidentified.

In our study, all dogs with IMHA had platelet counts ≥ 63,000 platelets/dL. Platelets have a major effect on whole blood hemostasis, and the platelet count will influence the K, α, MA, and G values. The same hypercoagulability demonstrated in the study dogs may not be evident in dogs with IMHA and platelet counts < 63,000 platelets/dL. Additional studies are needed to determine the overall hemostatic function in dogs with IMHA and concurrent moderate-to-severe thrombocytopenia.

The objective of the present study was to identify whether dogs with primary IMHA have hypercoagulability that is inherent to the disease process alone and not attributable to treatment. Thromboelastography was considered an adequate means of obtaining this information. However, the thromboelastography results are a reflection of their hemostatic condition at only 1 point in time; therefore, serial measurements of thromboelastography values in dogs with IMHA may provide a more dynamic representation of their coagulation state and the effect of treatments on hemostasis. On the basis of results from kaolin-activated thromboelasticity testing, dogs with primary IMHA and platelet counts ≥ 63,000 platelets/dL were in a hypercoagulable state at the time of diagnosis, upon admission to the hospital, and prior to immunosuppressive treatment, blood transfusions, or anticoagulant administration. Additional prospective studies are indicated to identify the relationship of thromboelastography findings with hypercoagulability and the development of thrombosis. Serial thromboelastographic measurements may be useful for future randomized controlled clinical trials to evaluate various anticoagulant and antiplatelet treatments and their effects on coagulation status, survival, and outcome.

References


From this month’s AJVR

Ultrasoundographic characterization of the liver, caudal vena cava, portal vein, and gallbladder in goats

Ueli Braun and Kathrin Steininger

Objective—To characterize the localization, visible extent (ie, measurement of selected dimensions), and appearance of the liver, caudal vena cava, portal vein, and gallbladder via ultrasonography in healthy goats.

Animals—27 female Saanen goats.

Procedures—A 5.0-MHz linear transducer was used to ultrasonographically examine the localization, visible extent of various dimensions, and appearance of the liver, caudal vena cava, portal vein, and gallbladder from the right side of each goat.

Results—Images of the liver were obtained in all goats. The dorsal margin of the liver extended in a cranioventral to caudodorsal direction parallel to the caudal margin of the lungs. The greatest visible extent of the liver was evident at the seventh and eighth intercostal spaces (mean value, 15.9 cm), and width was evident at the 10th intercostal space (mean value, 5.2 cm). The caudal vena cava had a triangular shape on cross section; the maximum width in cross section, circumference, and surface area ranged from 1.2 to 1.8 cm, 4.8 to 5.2 cm, and 0.8 to 1.1 cm², respectively. The portal vein was round on cross section (diameter, 0.8 to 1.7 cm) with stellate ramifications into the liver parenchyma. The gallbladder was pear-shaped and variable in size; it extended beyond the ventral margin of the liver to a variable degree depending on the amount of bile.

Conclusions and Clinical Relevance—Results provided information regarding the ultrasonographic appearance of the liver, caudal vena cava, portal vein, and gallbladder in healthy goats; these data may be useful during examination of goats with suspected liver disease. (Am J Vet Res 2011;72:219-225)