Evaluation of thromboelastography for prediction of clinical bleeding in thrombocytopenic dogs after total body irradiation and hematopoietic cell transplantation

Melissa C. Bucknoff, DVM; Rita M. Hanel, DVM; Steven L. Marks, BVSc, MS; Alison A. Motsinger-Reif, PhD; Steven E. Suter, VMD, PhD

Objective—To determine whether thromboelastography is more accurate than conventional methods of evaluating hemostasis for the prediction of clinical bleeding in thrombocytopenic dogs following total body irradiation (TBI) and bone marrow transplantation (BMT).

Animals—10 client-owned thrombocytopenic dogs with multicentric lymphoma.

Procedures—Results of a kaolin-activated thromboelastography assay, platelet count, and buccal mucosal bleeding time were evaluated for correlation to clinical bleeding.

Results—Maximum amplitude, derived via thromboelastography, was the only hemostatic variable with significant correlation to clinical bleeding. Buccal mucosal bleeding time had a high sensitivity but poor specificity for identifying dogs with clinical bleeding.

Conclusions and Clinical Relevance—Compared with buccal mucosal bleeding time and platelet count, thromboelastography was more reliable at identifying thrombocytopenic dogs with a low risk of bleeding and could be considered to help guide the use of transfusion products in dogs undergoing TBI and BMT. (Am J Vet Res 2014;75:425–432)

Thrombocytopenia is a common abnormality in veterinary patients and an important cause of mucosal bleeding. The ability to correlate results of hemostatic assays to clinical phenotypes in such patients is an emerging topic of exploration. Conventional methods of predicting the risk of bleeding in thrombocytopenic veterinary patients consist of an absolute platelet count and tests of primary hemostasis, such as determination of BMBT. Thromboelastography and rotational thromboelastometry are diagnostic tools that use the viscoelastic properties of clot formation to provide an assessment of global coagulation, thereby identifying both hyper- and hypocoagulable states. In human medicine, thromboelastography and rotational thromboelastometry are used to monitor and guide transfusion therapy in patients with trauma-associated coagulopathy and those undergoing cardiac bypass or liver transplantation. Thromboelastography has also been used to monitor therapeutic efficacy of platelet transfusions in patients with chemotherapy-induced thrombocytopenia. In veterinary medicine, several studies have used thromboelastography as a means of assessing global hemostatic function in both healthy dogs and those with a multitude of diseases causing hypo- and hypercoagulable states, including parvovirus enteritis, protein-losing nephropathy, neoplasia, immune-mediated hemolytic anemia, and disseminated intravascular coagulation. However, the authors are aware of only 1 study evaluating the correlation of thromboelastography to clinical bleeding in dogs and are not aware of any studies evaluating the correlation of thromboelastography variables to clinical bleeding in dogs with thrombocytopenia as the primary contributor to abnormal hemostasis.

Immune-mediated thrombocytopenia is the most common disease in small animal veterinary medicine requiring blood transfusions as a result of thrombocytopenia. However, causation, onset, and progression

ABBREVIATIONS

aPTT Activated partial thromboplastin time
BMBT Buccal mucosal bleeding time
BMT Bone marrow transplant
IMTP Immune-mediated thrombocytopenia
MA Maximum amplitude
PT Prothrombin time
ROC Receiver operating characteristic
TBI Total body irradiation
of the disease are variable, making thrombocytopenia associated with IMTP a difficult variable to isolate and study. Total body irradiation is an iatrogenic cause of bone marrow suppression and thrombocytopenia. After TBI, thrombocytopenia predictably occurs in 7 to 10 days,21 in accordance with the typical lifespan of canine circulating platelets.21,22 For this reason, dogs with B- or T-cell lymphoma undergoing autologous BMT following TBI provide a unique opportunity for evaluating hemostatic function.

The primary objective of the study reported here was to determine whether thromboelastography is more accurate than conventional methods of evaluating hemostasis for the prediction of clinical bleeding in thrombocytopenic dogs. Secondary objectives were to assess the correlation of clinically applicable hemostatic variables, specifically platelet count, BMBT, and thromboelastography-derived MA, with mucosal bleeding and spontaneous clinical bleeding events (melena, hematochezia, epistaxis, or hematuria) in a relatively uniform cohort of dogs with a known etiology for their thrombocytopenia. Other objectives included the evaluation of other readily available clinical and laboratory variables and their correlation with mucosal bleeding and clinical bleeding events as well as the analysis of platelet count and its association with thromboelastography variables. Our hypothesis was that MA would be more accurate than BMBT or platelet count at predicting clinical bleeding in thrombocytopenic dogs following TBI and BMT.

Materials and Methods

Dogs—Ten client-owned dogs with multicentric lymphoma sequentially enrolled into the canine BMT program from July 2009 through February 2010 were studied. Breeds included 2 Boxers, 3 German Shepherd Dogs, and 1 each of the following: Treeing Walker Coonhound, Labrador Retriever, Rhodesian Ridgeback, Chesapeake Bay Retriever, and Vizsla. There were 6 castrated males, 2 sexually intact males, and 2 spayed females. The median body weight was 39 kg (range, 25.6 to 55.6 kg), and the median age was 4.5 years (range, 3 to 10.25 years). Peripheral blood mononuclear cell aphereses were performed with a cell separator as described.21 This was followed by lethal TBI and infusion of the harvested mononuclear cells. The daily blood analyses and intermittent coagulation monitoring were performed as part of the program's protocol to aid in the clinical evaluation of these patients. Informed consent was obtained regarding the risks associated with TBI, apheresis, associated diagnostic testing, monitoring, and BMT.

Design—The study was a prospective observational cohort study, with mucosal bleeding and clinical bleeding as the examined attributes. Clinical bleeding included episodes of melena during defecation, hematochezia, epistaxis, or hematuria. Dogs were considered to have minor mucosal bleeding if new-onset petechiae or ecchymoses were detected at the time of physical examination. If a dog had evidence of both mucosal bleeding and clinical bleeding at the same time point, the data were grouped according to the more severe bleeding event. For example, if a dog had both new petechiae and epistaxis, the event was considered clinical bleeding only for that 24-hour period. If new-onset mucosal bleeding or clinical bleeding occurred the following day, it was a separate event. If none of the aforementioned was present, the category of no bleeding was assigned. The same 2 investigators performed CBC, serum biochemical analyses, and physical examination daily on all dogs in accordance with the established BMT protocol. Clinical signs consistent with clinical bleeding or mucosal bleeding, as described, were recorded. Hemostasis monitoring was performed at hospital admission, when the dog had a platelet count ≤ 30,000 platelets/µL or ≤ 10,000 platelets/µL and a rebound platelet count ≥ 10,000 platelets/µL, as well as within 12 hours before or after a clinical bleeding event but prior to the administration of any blood products. If a dog received a blood product, any information gathered within 48 hours after the transfusion was excluded. Coagulation monitoring included dual-channel kaolin-activated thromboelastography and determination of BMBT, PT, aPTT, D-dimer concentration, and fibrinogen concentration.

Sample collection and preparation—Blood was collected by either jugular or saphenous venipuncture with an 18- or 21-gauge needle or through a centrally placed jugular or saphenous catheter. Central lines were placed in all dogs at the time of TBI; thus, venipuncture samples were primarily used for admission samples. All blood samples were collected from the central venous catheter in accordance with a described method.23 Briefly, the infusion line of the catheter was clamped close to the sample collection port at the central catheter hub, and 6 mL of blood was removed and reserved as a purge sample. Another syringe was used to collect the sample used for thromboelastography, standard hemostasis analysis, and serum biochemical analysis or CBC. Blood samples were collected into plain tubes, citrated tubes, and EDTA tubes, in that order, depending on diagnostic needs of the dog at the time of collection. The 2.7-mL citrated tubes and EDTA tubes were inverted several times after sampling to ensure mixing of 3.2% trisodium citrate and blood in a 1:9 ratio and 3.6 mg of EDTA and blood, respectively. Samples obtained for thromboelastography were kept upright at room temperature (23°C) for 30 minutes prior to analysis. All other samples were immediately submitted to the clinical pathology laboratory. In the event that the clinical pathology laboratory was not available, the emergency service laboratory processed the samples.

Thromboelastography analysis—The computerized thromboelastography analysis was performed in duplicate, by 1 of 2 operators, according to the manufacturer's instructions.24 Each standard thromboelastography pin and cup were placed in the 37°C prewarmed instrument holder, and 20 µL of calcium chloride was pipetted into the cup. After 30 minutes at 23°C, 1 mL of canine citrated blood was placed in a commercial vial containing kaolin, buffered stabilizers, and a blend of phospholipids and inverted 5 times. Then, 340 µL of kaolin-activated citrated blood was pipetted into the cup within 1 minute for a total volume of 360 µL. The thromboelastography analyses were run for a minimum of 60 minutes, and the following values were recorded: R (reaction time), K (clot formation time), α-angle (α...
measure of the rapidity of clot formation), MA (mm), and G (global clot strength or clot shear elastic modulus [dyn/cm²]). The MA was used to derive G as follows:

\[ G = 500 \times MA/(100 - MA) \]

The contributions from hemostatic components to each thromboelastography variable have been described.\(^\text{11,12}\) BMBT—Each BMBT test was performed cage-side using a standardized fully automated incision device\(^\text{e}\) by 1 of 2 operators, and all dogs were awake and in lateral recumbency, with minimal restraint. The upper lip was outwardly exposed by use of gauze tied over the bridge of the nose. A spring-activated surgical steel blade housed in a plastic cartridge, from which it protruded and retracted automatically, was positioned and discharged over the buccal surface of the lip, creating a minor, standardized incision with a depth of 1.0 mm and width of 5.0 mm. Blood was wicked away from the site with filter paper, with care taken not to disturb the forming clot and platelet plug. Bleeding time was measured with a stopwatch. The established maximum reference limit for time to clot formation of 4.2 minutes was used.\(^\text{27}\) A maximum cutoff of 10 minutes was used if a clot had not formed.

Tests of hemostasis—The coagulation profile included aPTT, PT, fibrinogen concentration, D-dimer concentration, and platelet count. The PT and aPTT were determined with a electromechanical clot detection method performed by use of a heparinase analyzer\(^\text{i}\) with thromboplastin\(^\text{g}\) and cephalin with plasma activator\(^\text{h}\) reagents for PT and aPTT, respectively. Concentration of D-dimer was determined by use of a latex autoagglutination method.\(^\text{j}\) Platelet concentration was determined by use of an automated hematology analyzer\(^\text{l}\) and results were verified by visual inspection. Plasma fibrinogen concentration was determined by a heat precipitation method.\(^\text{28}\) Platelet counts conducted after normal business hours were determined by the emergency laboratory automated analyzer\(^\text{a}\) and were verified the next business day by the clinical pathology laboratory. After normal business hours, an aPTT and PT were determined with an automated cage-side monitor\(^\text{b}\) by 1 of 2 operators. This method is markedly different in sensitivity from the electromechanical clot detection method. However, this study was observational and results were provided by the method available at the time of request. Only 3 samples of the 43 reported data sets were collected and processed after normal business hours.

Statistical analysis—Data recorded for analysis at each time point included rectal temperature, heart rate, R, K, α-angle, MA, G, platelet count, BMBT, fibrinogen concentration, D-dimer concentration, PT, aPTT, PCV, and total solids concentration. D-dimer concentrations were assessed semiquantitatively and assigned an ordinal designation according to the following scale: 0 = < 250 ng/mL, 1 = 250 to 500 ng/mL, 2 = 501 to 1,000 ng/mL, 3 = 1,001 to 2,000 ng/mL, and 4 = > 2,000 ng/mL. Because PT and aPTT data were obtained by 2 methodologies with different ranges, data were normalized by dividing the actual result by the upper reference limit for that methodology.

Prior to analysis, any biases in thromboelastography measurement, such as by ordering or channel, were tested for association with the thromboelastography variables. Trend tests were performed to test for such batch effects, and for each test, no significant (\( P > 0.05\)) effects were detected. This was an important quality control step to prevent spurious associations in the primary analyses.

The primary objective was to evaluate associations between thromboelastography-derived MA, BMBT, and platelet count and bleeding events (clinical, mucosal, or no bleeding). If a dog had mucosal bleeding and clinical bleeding at the same time point, the event was categorized as clinical bleeding to provide analysis for the more severe bleeding event. Thus, the same dog was never included in 2 groups at the same time, and analyses were performed with data grouped according to events (clinical, mucosal, or no bleeding), and not dogs. Because this was a longitudinal, prospective study, association analyses were performed for each variable by Cox proportional hazards analysis with a time to bleeding event component. The analysis was repeated for several contrasts: no bleeding or mucosal bleeding versus clinical bleeding, mucosal bleeding versus no bleeding, clinical bleeding versus mucosal bleeding, and clinical bleeding versus no bleeding. All analyses were performed with statistical software.\(^\text{26}\) A Bonferroni correction was applied to correct for the number of hypotheses established for the primary objective of the study (testing for associations between the 3 groups and the hemostatic variables). This correction approach is a conservative approach when variables are associated. The \( P \) values were corrected for multiple comparisons, and values of \( P \leq 0.05 \) after correction were considered significant. Additionally, the thromboelastography variables were tested for association with platelet count by means of linear regression.

For the secondary objectives or for associations between remaining recorded variables and bleeding groups, \( P \) values were not corrected for multiple comparisons because of the exploratory nature of these objectives. However, the \( P \) values were also not significant, obviating a need for correction. Correction was not made for the number of contrasts performed, but instead only for the number of primary variables tested, because of the exploratory nature of the analysis.

To evaluate the predictive performance of significant associations detected in the first stage of analysis, ROC curve analysis was performed to calculate and test the area under the curve. The ROC curve analysis was performed with a Web-based calculator.\(^\text{29}\) Hypothesis testing to evaluate the significant prediction after ROC curve analysis was performed by means of the method described by DeLong et al.\(^\text{29}\)

Results

Ten dogs were studied, which generated 50 data collection points. Seven time points were excluded: 3 due to temporal association with a transfusion product and 2 due to inappropriate sample acquisition. The
remaining 43 data collection points were summarized (Table 1). All 10 dogs had thrombocytopenia with platelet counts of ≤ 10,000 platelets/µL following TBI, and all dogs had a rebound in platelet count to ≥ 10,000 platelets/µL prior to hospital discharge (Figure 1). Two of the dogs were thrombocytopenic on admission, and all pretreatment samples were nonbleeding events. Four dogs received a transfusion of fresh blood, which was irradiated prior to administration, during hospitalization. The decision to transfuse was at the discretion of the primary clinician responsible for each case. Nine dogs survived to hospital discharge, and 1 dog died. That dog developed acute pancreatitis and aspirated vomitus, which precipitated its death.

**Bleeding events**—Six of the dogs had ≥ 1 clinical bleeding event, for a total of 7 events, which included melena (n = 2), epistaxis (4), hematuria (2), and hematemesis and regurgitation (1). Mucosal surface bleeding at the predetermined collection points occurred in 3 dogs for a total of 4 events. All dogs that had clinical bleeding events also had at least 1 mucosal bleeding event. If a dog had a mucosal and clinical bleeding event at the same time point, it was categorized as clinical bleeding to provide analysis for the more severe bleeding event. Four dogs did not have any signs of mucosal or clinical bleeding. The clinical bleeding events occurred over a mean of 10 days following TBI (Figure 1).

**Thromboelastography and coagulation profile results**—Hemostatic variables were measured and compared among groups. Median and range values for all variables are reported (Table 1). The median platelet count was 6,000 platelets/µL during clinical bleeding events and 11,000 platelets/µL during mucosal bleeding events. In 7 of 7 clinical bleeding events and 4 of 4 mucosal bleeding events, a platelet count < 30,000 platelets/µL was documented. A platelet count < 30,000/µL was also present in 20 of 32 events with no bleeding. Six of 7 clinical bleeding events and 4 of 4 mucosal bleeding events had a prolonged BMBT (> 4.2 minutes). In 19 of 32 events with no bleeding, a prolonged BMBT was also present. Overall, BMBT was prolonged in 30 of 50 time points. Coagulopathy, as defined by PT or aPTT prolongation during a clinical bleeding event, was detected in 2 dogs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No bleeding (n = 32)</th>
<th>Mucosal bleeding (n = 4)</th>
<th>Clinical bleeding (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (min)</td>
<td>4.9 (1.8–10.8)</td>
<td>3.5 (2.8–4.6)</td>
<td>4.6 (2.8–11.2)</td>
</tr>
<tr>
<td>K (min)</td>
<td>2.4 (0.8–11.7)</td>
<td>2.8 (1.3–3.7)</td>
<td>2.5 (1.4–17.5)</td>
</tr>
<tr>
<td>α-Angle (°)</td>
<td>59.2 (21.5–88.3)</td>
<td>62.4 (60.2–71.5)</td>
<td>52.5 (78–69.5)</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>56.5 (24.6–77.1)</td>
<td>42.5 (36.2–69.7)</td>
<td>37.6 (10.1–50.4)</td>
</tr>
<tr>
<td>G (dyn/s)</td>
<td>5.9 (1.7–16.9)</td>
<td>6.6 (5.0–11.5)</td>
<td>3.1 (0.6–5.1)</td>
</tr>
<tr>
<td>Platelet count (× 10^9/µL)</td>
<td>20.5 (4.0–44.8)</td>
<td>11.5 (8.0–38.0)</td>
<td>6.0 (2.0–14.0)</td>
</tr>
<tr>
<td>BMBT (min)</td>
<td>6.1 (1.24–10.0)</td>
<td>8.2 (2.1–10)</td>
<td>&gt; 10 (8.16–10.0)</td>
</tr>
<tr>
<td>PT ratio</td>
<td>0.82 (0.71–1.01)</td>
<td>0.84 (0.81–0.98)</td>
<td>0.87 (0.76–0.94)</td>
</tr>
<tr>
<td>aPTT ratio</td>
<td>0.92 (0.59–1.17)</td>
<td>0.9 (0.79–1.37)</td>
<td>1.0 (0.78–2.42)</td>
</tr>
<tr>
<td>D-dimer (ng/mL)</td>
<td>&lt; 250 (&lt; 250–1,000)</td>
<td>&lt; 250 (&lt; 250–1,000)</td>
<td>&lt; 250 (&lt; 250–1,000)</td>
</tr>
<tr>
<td>Fibrinogen (mg/mL)</td>
<td>400 (100–600)</td>
<td>400 (300–400)</td>
<td>350 (300–500)</td>
</tr>
<tr>
<td>WBC count (× 10^6/µL)</td>
<td>4,175 (1,070–53,600)</td>
<td>6,005 (1,090–9,450)</td>
<td>1,440 (200–3,300)</td>
</tr>
<tr>
<td>RBC count (× 10^6/µL)</td>
<td>3.9 (3.0–5.1)</td>
<td>3.5 (2.7–3.7)</td>
<td>3.8 (2.7–4.8)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>34 (20–51)</td>
<td>31.5 (27–41)</td>
<td>29 (15–38)</td>
</tr>
<tr>
<td>Total solids (g/dL)</td>
<td>6.0 (4.6–8.0)</td>
<td>4.9 (4.8–6.5)</td>
<td>5.4 (4.0–6.5)</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.5 (36.3–39.72)</td>
<td>38.5 (37.94–38.83)</td>
<td>38.3 (36.5–39.39)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>103 (70–156)</td>
<td>95 (80–153)</td>
<td>100 (80–122)</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>56.5 (24.6–77.1)</td>
<td>43.5 (36.2–69.7)</td>
<td>37.6 (10.1–50.4)</td>
</tr>
<tr>
<td>α-Angle (°)</td>
<td>59.2 (21.5–88.3)</td>
<td>62.4 (60.2–71.5)</td>
<td>52.5 (78–69.5)</td>
</tr>
<tr>
<td>K (min)</td>
<td>2.4 (0.8–11.7)</td>
<td>2.8 (1.3–3.7)</td>
<td>2.5 (1.4–17.5)</td>
</tr>
<tr>
<td>R (min)</td>
<td>4.9 (1.8–10.8)</td>
<td>3.5 (2.8–4.6)</td>
<td>4.6 (2.8–11.2)</td>
</tr>
<tr>
<td>G (dyn/s)</td>
<td>5.9 (1.7–16.9)</td>
<td>6.6 (5.0–11.5)</td>
<td>3.1 (0.6–5.1)</td>
</tr>
<tr>
<td>Platelet count (× 10^9/µL)</td>
<td>20.5 (4.0–44.8)</td>
<td>11.5 (8.0–38.0)</td>
<td>6.0 (2.0–14.0)</td>
</tr>
<tr>
<td>BMBT (min)</td>
<td>6.1 (1.24–10.0)</td>
<td>8.2 (2.1–10)</td>
<td>&gt; 10 (8.16–10.0)</td>
</tr>
<tr>
<td>PT ratio</td>
<td>0.82 (0.71–1.01)</td>
<td>0.84 (0.81–0.98)</td>
<td>0.87 (0.76–0.94)</td>
</tr>
<tr>
<td>aPTT ratio</td>
<td>0.92 (0.59–1.17)</td>
<td>0.9 (0.79–1.37)</td>
<td>1.0 (0.78–2.42)</td>
</tr>
<tr>
<td>D-dimer (ng/mL)</td>
<td>&lt; 250 (&lt; 250–1,000)</td>
<td>&lt; 250 (&lt; 250–1,000)</td>
<td>&lt; 250 (&lt; 250–1,000)</td>
</tr>
<tr>
<td>Fibrinogen (mg/mL)</td>
<td>400 (100–600)</td>
<td>400 (300–400)</td>
<td>350 (300–500)</td>
</tr>
<tr>
<td>WBC count (× 10^6/µL)</td>
<td>4,175 (1,070–53,600)</td>
<td>6,005 (1,090–9,450)</td>
<td>1,440 (200–3,300)</td>
</tr>
<tr>
<td>RBC count (× 10^6/µL)</td>
<td>3.9 (3.0–5.1)</td>
<td>3.5 (2.7–3.7)</td>
<td>3.8 (2.7–4.8)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>34 (20–51)</td>
<td>31.5 (27–41)</td>
<td>29 (15–38)</td>
</tr>
<tr>
<td>Total solids (g/dL)</td>
<td>6.0 (4.6–8.0)</td>
<td>4.9 (4.8–6.5)</td>
<td>5.4 (4.0–6.5)</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.5 (36.3–39.72)</td>
<td>38.5 (37.94–38.83)</td>
<td>38.3 (36.5–39.39)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>103 (70–156)</td>
<td>95 (80–153)</td>
<td>100 (80–122)</td>
</tr>
</tbody>
</table>

*Within a row, values with the same superscript letter indicate a significant (P < 0.05) correlation between bleeding categories. G = Global clot strength. K = Clot formation time. R = Reaction time. The PT and aPTT data were normalized by dividing the actual result by the upper limit of the reported reference range.
Abnormal values for thromboelastography variables were common (Table 1). Linear regression analysis revealed that all thromboelastography variables were nominally associated with platelet count, and MA, α-angle, and G were significantly associated with platelet count even after Bonferroni corrections (Table 2).

At 1 time point each, with values of 2.4 and 1.3 times the upper limit of the reference range.

Abnormal values for thromboelastography variables were common (Table 1). Linear regression analysis revealed that all thromboelastography variables were nominally associated with platelet count, and MA, α-angle, and G were significantly associated with platelet count even after Bonferroni corrections (Table 2).

Associations among no bleeding, mucosal bleeding, and clinical bleeding—In comparison of data collected for dogs that had mucosal or no bleeding to those for dogs that had clinical bleeding, there was a significant ($P = 0.048$) association with respect to MA (Figure 2). In comparison of data collected for dogs that had mucosal bleeding to those that had no bleeding, no significant association was found with any variable. In comparison of data collected for dogs with clinical bleeding to those with mucosal bleeding, there was a significant ($P = 0.047$) association with respect to MA. In comparison of data collected for patients with clinical bleeding to those with no bleeding, there was a significant association with respect to MA ($P = 0.010$) and G ($P = 0.034$).

ROC curve analysis—An ROC curve analysis was performed to assess the predictive performance of MA for bleeding events. Several comparisons were performed, contrasting the magnitude of bleeding events in paired analyses. In comparison of mucosal or no bleeding (considered true negatives) to clinical bleeding (considered true positives), the area under the curve was 0.813 and bootstrap testing for comparison of this AUC to the null yielded $P < 0.05$. Setting an MA cutoff value of 50 mm, the specificity would be 90% and the sensitivity would be 59%. The hazards ratio for dogs with an MA less than this cutoff value to greater than this cutoff value would be 8.59.

In this cohort of thrombocytopenic dogs with lymphoma that underwent TBI and BMT, the MA derived by kaolin-activated thromboelastography was the only hemostatic variable that consistently correlated with signs of spontaneous clinical bleeding. However, the predictive power of this test to discern dogs that would develop bleeding from those that would not was poor. Conversely, the MA does appear to be useful in identifying dogs with a low risk for bleeding. The specificity of identifying these low-risk dogs was approximately 90% when an MA cutoff of 50 mm was used with kaolin-activated thromboelastography.

Thrombocytopenia secondary to irradiation was presumed to be the major hemostatic derangement responsible for clinical bleeding in this cohort of dogs. Values in the reference range for PT and aPTT at admission in all dogs excluded the most common inherited coagulopathies, such as hemophilia A or B. However, subtle coagulation factor deficiencies that may have affected thromboelastography results without prolonging the PT or aPTT were not ruled out, given that this study was observational and individual coagulation factor concentrations were not determined. A consumptive coagulopathy secondary to hemorrhage was unlikely, considering that platelet counts decreased prior to documented bleeding and all bleeding events were mild. A consumptive coagulopathy such as disseminated intravascular coagulation was possible, given that all dogs were transiently leukopenic secondary to irradiation. For this reason, complete coagulation profiles, including aPTT, PT, D-dimer concentration, and fibrinogen concentration, were monitored. In the clinical bleeding group, no dogs had a prolonged PT, and 2 dogs had prolonged aPTTs of 2.4 and 1.3 times the upper limit of the reference range. In the first dog, D-dimer concentration was increased (500 to 1,000 ng/dL), with a fibrinogen concentration of 300 mg/mL. This was also the nonsurvivor. The second dog had an undetected
D-dimer concentration (<250 ng/mL) and a fibrinogen concentration of 400 mg/mL. There was also no clinical evidence of a coagulopathy, such as body cavity hemorrhage, in any dog. Thus, with the exception of 1 dog, the contribution of disseminated intravascular coagulation as defined by abnormalities in PT, aPTT, D-dimer concentration, platelet count, and fibrinogen concentration was minimal. Hence, it is reasonable to conclude that irradiation-induced thrombocytopenia was the major contributing factor with regard to hemostatic complications. However, neither platelet count nor BMBT were significantly correlated with hemostatic bleeding. Tests that reliably predict spontaneous bleeding in humans and other animals are lacking. In veterinary medicine, 2 reports include discussion of the use of platelet counts and coagulation profiles in relation to bleeding following biopsy. In dogs, both platelet count (<80,000 platelets/µL) and PT had an association with bleeding, which was more common in dogs that had renal biopsies. A retrospective study of complications of renal biopsies noted an increased percentage of severe bleeding in dogs and cats with abnormal coagulation profiles. In humans, coagulation profiles and platelet counts are not predictive of bleeding events, a finding that precipitated the search for other monitoring tools, such as thromboelastography. One study in veterinary medicine prospectively evaluated how tissue factor–activated thromboelastography correlated in veterinary medicine prospectively evaluated how tissue factor–activated thromboelastography correlated in clinical bleeding in dogs, compared with a routine coagulation profile. Thromboelastography correctly identified dogs with clinical bleeding with a positive predictive value of 89% and a negative predictive value of 98%, whereas coagulation profiles had a positive predictive value between 50% and 81% and a negative predictive value between 92% and 93%, depending on the observer. Underlying disease processes were varied, as was the underlying hemostatic abnormality contributing to blood loss. The present study evaluated spontaneous bleeding resulting from predictable thrombocytopenia, but results were similar in that thromboelastography was better at identifying dogs with a low risk of bleeding.

In humans, thromboelastography- and rotational thromboelastometry–based algorithms are used to monitor and guide transfusion therapy in patients with trauma–associated coagulopathy and those undergoing cardiac bypass or liver transplantation. This has resulted in considerable reductions in the number of blood products administered, which has theoretical benefits with regard to the development of complications such as transfusion–related acute lung injury and circulatory overload as well as the potential for infectious complications and increased cost. In the cohort of dogs in the present study, severe clinical bleeding requiring transfusion products was minimal, and no dog had hemodynamic instability secondary to blood loss. However, this is not always the case with pathological causes of thrombocytopenia, and there is still controversy surrounding what the appropriate trigger should be for platelet product administration. In human medicine, the debate continues about whether prophylactic platelet transfusions should be given or reserved specifically for therapeutic administration. This ongoing controversy is likely due to the lack of specificity of current methods, such as platelet count, to appropriately recognize the need for or guide the usage of blood products. The use of algorithms that incorporate tests of platelet number and global hemostatic function should help bridge the gap in consensus surrounding this issue. Viscoelastic testing may arguably fill this void. By evaluating the patient’s ability to form a stable clot through incorporation of the patient’s own plasma and platelets, with minimal manipulation, a more physiologic picture may be presented.

This study also documented the association of platelet count with the thromboelastography variables MA, G, and α-angle. This has been determined in multiple human studies with thrombocytopenia secondary to chemotherapy, dilutional models, or models manipulating platelet counts by means of immunomagnetic separation of platelets. There is typically a strong positive correlation between MA (thromboelastography), or maximum clot firmness (rotational thromboelastometry), and platelet count. Correlations between kinetics of clot formation (α-angle and K) also exist, but the relationship with R is poor. This helps to explain the strong correlation in the present study between bleeding and MA, considering that thrombocytopenia was the primary hemostatic derangement. However, the ability of thromboelastography to evaluate global hemostatic function likely accounts for its ability to better discern clinical bleeding, compared with platelet count alone.

In a recent prospective pilot study evaluating hypercoagulability in dogs with IMTP, thromboelastography was used to detect hypercoagulability in dogs with rebounding platelet counts. The authors found evidence of hypercoagulability in all dogs with rebounding platelet counts >40,000 platelets/µL. These data cannot be directly compared with the present study because the highest rebound platelet count analyzed prior to discharge or death was 36,000 platelets/µL and the cause of the thrombocytopenia was different. However, the presence of hypercoagulability in dogs with IMTP supports the theory that immature platelets may be more reactive than mature ones. In the present study, the lack of clinical bleeding events, despite severe thrombocytopenia, may indicate the possibility of platelet hyperactivity attributable to the immature platelets produced by the bone marrow during the recovery phase following BMT. Further studies are needed to investigate this theory.

Limitations of this study included the small sample size and the small number of dogs with bleeding events. Additionally, statistics were not strictly corrected for all the contrasts performed. The analysis was stratified into 2 stages: a primary analysis that was planned and was conservatively controlled for multiple testing and a secondary analysis that was more exploratory. Because of this, the type I error of the primary analysis was conservatively controlled, but the type I error of the secondary results were not tightly controlled. For the secondary analyses, if positive findings were present, they were to
be viewed as more hypothesis generating than strictly hypothesis testing. No correlations were present in the secondary analysis. However, the consistency of the MA association across multiple contrasts in the primary analysis provided strong support for the correlation.

One must also consider whether anemia influenced the results, given that it contributes to relative hypercoagulability. The median PCV for the clinical bleeding group was 29%, which was the lowest of the 3 groups. In a recent study evaluating the effect of Hct, platelet count, and coagulation factors on thromboelastometry in dogs, the authors found a significant correlation between all values derived via rotational thromboelastometry and Hct. These dogs had Hct values within the reference range. Yet, those with lower Hct had relatively hypercoagulable thromboelastometry results, compared with dogs with higher Hct. Thus, it is possible that our results may have been skewed in the hypercoagulable direction, thereby masking a better relationship between thromboelastography results and bleeding events. However, no statistical association was found between PCV and bleeding tendency among the different groups in this study and thus application of correction formulas was not attempted.

The effect of preanalytic and analytic variables on thromboelastography must also be considered. The thromboelastography was performed by 1 of 2 operators with a strong activator (kaolin). No gross hemolysis was reported in any sample, and venipuncture, when performed, was without complication. Blood samples in 9 of 10 dogs were collected via jugular venipuncture on admission. The remaining blood samples were collected via centrally placed venous catheters, with the exception of 6 samples collected via venipuncture, by use of a syringe and 20-gauge needle, from the lateral saphenous vein. The authors of a recent report comparing venous sampling methods for thromboelastography in clinically healthy dogs found no significant difference in MA as obtained via these 3 methods. Overall, there was minimal effect of these variables on the primary outcome.

The cohort of dogs in the present study was selected because of their predictable thrombocytopenia. However, although the homogeneity of the hemostatic abnormalities induced in these dogs was ideal from a research standpoint, this limits its applicability to cases of thrombocytopenia due to other etiologies or in dogs with multiple hemostatic abnormalities. Thromboelastography variables are reflective of global hemostatic function because of their codendence on multiple hemostatic variables. Maximum clot firmness, similar to MA, has a strong correlation with platelet count and fibrinogen concentration, and our cohort of dogs had mostly fibrinogen concentrations within reference range. Thus, goals for future studies would be to evaluate the correlation of hemostatic assays with bleeding in dogs with abnormal fibrinogen concentrations and disease states, such as IMTP, or in dogs with multiple hemostatic abnormalities. The efficacy of various transfusion products on these same variables should also be prospectively evaluated.

The kaolin-activated thromboelastography–derived variable MA was the only hemostatic variable that was consistently associated with signs of spontaneous clinical bleeding. The BMBT, although able to be determined with a convenient cage-side test for alterations in platelet number and function, is extremely sensitive and not specific enough to aid in decisions regarding risk of bleeding. Although further studies are needed in a more varied patient population, it appears that thromboelastography may be valuable in discerning dogs with a low risk of bleeding and could be useful in the derivation of transfusion algorithms for thrombocytopenic dogs.

References

10. Apleseht TO, Bruserud O, Wentzel-Larsen T, et al. Therapeuti...


