“Once upon a time, a bleeding dog walked in the door…”

This is a familiar opening line to many a story told down through the ages, from veterinary clinic to veterinary clinic, and so begins the search for the happy ending—in this case, not a glass slipper but the elusive blood clot.

Bleeding can occur in a variety of situations and due to a variety of causes. There is the tragic (but at least obvious-to-figure-out) trauma-induced “hit by car” dog who is rushed into the clinic in the owner’s arms. For every easy to comprehend case, there are an equal number of animals who present with bruises, bleeding abdomens, or trickles of blood from their noses who are complex puzzles and require a longer storyline to figure out the ending.

Before we, the veterinary knights in shining armor, can make the bleeding stop, we have to know why it started in the first place. And so, the story of bleeding begins…

Pathophysiology of Bleeding

Hemostasis, “the arrest of bleeding,” involves the interaction of an injured blood vessel, platelets, and clotting factors. There is a primary hemostasis phase involving the blood vessel’s interaction with platelets and a secondary hemostasis phase involving coagulation factors. A solid clot composed of platelets fused together and enmeshed in fibrin web is the end product of hemostasis. When a blood vessel is injured in the body, it is important that the blood loss be stopped quickly, that clotting only occur in injured vessels, and that the clot be dissolved quickly once the vessel has been repaired.

Primary Hemostasis:

- Local vascular contraction
- Platelet plug formation

When injury occurs to a blood vessel, the first slowing of blood results from constriction of the vessel. Vascular spasm is mediated by nervous reflexes from the injured vessel and nearby tissues, local muscle spasm, and factors released from platelets and traumatized tissues. Platelets coming into contact with a traumatized vessel wall’s exposed subendothelial collagen will adhere and change. The exposed tissue contains Von Willebrand’s Factor (a.k.a. Factor VIII:Ag or VWF) and fibronectin, contact proteins that activate the platelets and cause them to become “sticky.” The platelets bind to the subendothelial collagen, and their contractile proteins forcefully eject multiple active factors that promote further adhesion, shape change, and granule release in adjoining platelets. This successive attraction forms a platelet plug, which can stop blood loss by itself in small vessels or in larger vessels with small injuries. Platelets play an integral role in maintaining normal vascular integrity by closing the thousands of minute ruptures that occur daily in the body.

Secondary Hemostasis:

- Clotting of the plasma secondary to the interaction of coagulation factors

In larger vessels or more traumatized tissue, the fragile platelet plug forms a temporary seal, and a blood clot is required to stop the bleeding. Secondary hemostasis involves the formation of fibrin on the surface of the
platelet plug by coagulation factors. These factors, denoted by Roman numerals, circulate as inactive precursor forms of proteolytic enzymes. At the site of injury, they become converted to their active forms—the sequential activation of one factor leading to another is where the terminology of the “clotting cascade” originates. Although the coagulation cascade is often divided into an extrinsic, intrinsic, and common pathway—done in order to allow visualization of the clotting inside and outside the vascular system—these systems can and do often work together to trigger coagulation. The culmination of the activation and interaction of these clotting factors is the creation of a fibrin framework over the unstable initial platelet plug, i.e., the blood clot.

Fibrinolysis:

- The process whereby the blood clot is removed once the integrity of the blood vessel is restored

Once a blood clot is formed, it’s essential that it is eventually dissolved so that adequate blood flow can be reestablished. Plasmin is a proteolytic enzyme that digests fibrin fibers as well as Factors V, VIII, XII, and prothrombin and causes the eventual lysis of the blood clot. As fibrin is cleaved by plasmin, it leaves behind cross-linked fragments known as fibrin degradation products (FDPs).

This is the general concept of how bleeding stops when everything is working correctly.

BUT:

- Why is your patient bleeding? and…
- How do we get the bleeding to stop?

As the title of this talk is “cases of coagulopathies,” we are not going to be talking about situations where things have gone right but times when they have gone very wrong. As a technician, your role in figuring out the mystery of why your patient is bleeding is VITAL, and figuring out the backstory starts with the …

**Story Plot Points: History, Physical Exam, Diagnostic Tests**

**History:**

Obviously, as in the opening paragraph, if someone comes in and says “my dog has been hit by a car,” trauma is the why and now it is just figuring out the where and the how much. Unfortunately (or fortunately depending on your perspective), this is not the only bleeding scenario with which you will be presented. So it is good to ask questions, and this occurs before or during the physical exam, depending on the stability of the patient.

Questions to ask are as follows:

1. What is the gender and the age of your dog?
2. Any underlying medical problems? History of chronic disease?
3. Is your dog on any medications?
4. Any access to medications—either intentional or unintentional?
5. Does your dog live in a fenced yard, have the roam of the pasture, or is a welcome guest at the neighbors?
6. Does your dog have access to any known toxins?
7. What is your dog’s current vaccination history?
8. What is your dog’s travel history?
9. Has this situation ever happened before?
10. Do you know any of your pet’s siblings and have they had bleeding problems?
Physical Exam:

Once again, your role in gathering information for determining an underlying cause in a coaulopathy case is crucial. A quick but careful exam of the patient on intake—or while getting a TPR—can provide insight to the clinician and help direct and focus their time and energies.

In general, the animal who has a primary hemostatic disorder—involving the vascular endothelium, the platelet number, or function—will have evidence of “surface bleeding” such as petechiae. When platelets have failed to do their job and seal over small defects in capillaries that are traumatized through normal activity, tiny pinpoint hemorrhages will appear in the skin or mucous membranes. Blood in and around the eye can be visualized. Petechiae can coalesce into larger areas known as ecchymoses and typically occur on areas where pressure occurs (i.e., ventral chest, hip, etc.). If all the components of secondary hemostasis are intact, major hemorrhage is avoided.

In animals suffering from disorders of secondary hemostasis, bleeding into a body cavity is often seen. Hemothorax, hemoperitoneum, hemoarthrosis can all be seen as well as GI bleeding. Swollen joints, lameness, difficulty breathing, a distended abdomen with fluid wave, and melena can all potentially be seen in addition to extensive bruising with issues of secondary hemostatic disorders. As platelets can form the initial plug in the capillary on the skin surface, signs of a primary hemostatic disorder such as petechiae are typically not in evidence in secondary hemostatic dysfunction.

Several key points to remember on exam:

- A small amount of blood can cause major problems or significant clinical signs if it occurs in a tight space or a sensitive location: eyes, pericardium, brain, or spinal cord.

- Non-clotting blood that is obtained from the chest or abdominal cavity does NOT necessarily indicate a bleeding disorder. Blood accumulated in a body cavity for any length of time has often lost clotting factors and platelets due to the work of the fibrinolytic pathway and will not clot when removed via thoracocentesis or abdominocentesis.

- External hemorrhage can occur as a result of EITHER primary or secondary hemostasis and be manifest as epistaxis, melena, hematuria, oral bleeding, or hematemesis.

- Animals with severe defects in the secondary aspect of hemostasis can appear stable or clinically normal for long periods of time before showing clinical signs. In contrast, animals with defects of primary hemostasis such as thrombocytopenia will often show clinical signs early in their course of disease due to the fact that repair of minor damage in capillaries is needed as a daily process.

- CATS can be especially difficult creatures to diagnose early in the course of a severe secondary hemostatic defect and will often go undetected until major trauma or the accumulation of a major body cavity hemorrhage.

Diagnostic Tests to Evaluate Hemostasis:

Once a bleeding or bruised patient walks in the door, it is essential to determine the cause of the problem in order to apply appropriate treatment—anticoagulant rodenticide toxicity is treated very differently from immune-mediated thrombocytopenia. History and physical exam provide initial clues as to whether the cause of the bleeding is a failure of primary hemostasis (i.e., formation of a platelet plug) versus a secondary hemostatic or coagulopathic disorder. In-hospital tests such as a platelet count, BMBT, and ACT can provide a quick assessment for screening between a primary and secondary disorder.
Primary Hemostasis: Addressing Platelet Number and Function

The self-explanatory platelet count determines if thrombocytopenia is present; if not, then a BMBT in the face of a normal platelet typically indicates a platelet dysfunction or von Willebrand’s disease.

- **Platelet Number**

  Platelet numbers can be assessed either by automated hematology analyzers, by a manual counting technique, or by visually assessing a stained blood smear. Normal platelet counts will vary somewhat depending on the lab (*they will provide reference values*) but typically 150,000-400,000/ul are considered normal for a dog. When assessing a blood smear, 8-15 platelets should be seen per high powered oil emersion microscopic field.

- **Platelet Function**

  **BMBT: Buccal Mucosal Bleeding Time**

  If platelet count and vWf are normal, a prolonged BMBT indicates a platelet function defect. Disposable devices that make one to two standard 1 mm deep mucosal incisions are used for this test. Once this device makes a small standardized incision in the buccal mucosa, the time is measured until bleeding ceases.

  More extensive and elaborate testing of primary hemostasis such as assessment of platelet aggregation, adhesion and release can be done at specialized laboratories. These tests include PFA100 (platelet function analyzer), flow cytometric analysis of platelet products and surface markers, electron microscopy, platelet aggregation and nucleotide studies, and different testing modalities for von Willebrand’s factor (vWf).

Secondary Hemostasis: Assessing Coagulation

Coagulation screening tests for defects of the clotting cascade include the ACT, PT, and PTT.

- **ACT:** The activated clotting time (ACT) is sensitive to a deficiency or dysfunction of all the clotting factors (except Factor VII)—indicating problems with the intrinsic or common pathways. The factor deficiency must be less than 5% of normal to prolong the ACT; a prolonged ACT usually indicates a severe, clinically significant coagulopathy and the need for more sensitive tests to screen for disorders of coagulation. The ACT is determined by placing blood in a glass tube containing a contact activator and marking the time taken for blood to clot.

  Some hematologists argue that the ACT is, in essence, a less sophisticated APTT, and that if the ACT is prolonged, there is no reason to do the APTT. However, the ACT is a less sensitive test and since there is no phospholipid (platelet substitute) added to the ACT (the APTT includes this step), it is rare (but possible) to have a prolonged ACT due to severe and prolonged thrombocytopenia, while APTT is normal. As such, if there is clinical suspicion of a secondary bleeding disorder and the ACT is normal, the APTT must be performed.

- **PT and PTT:**
  - **PT:** The prothrombin time (PT) involves the use of a tissue thromboplastin reagent which activates factor VII it evaluates the extrinsic and common pathways.
  - **PTT:** The partial thromboplastin time (PTT) involves the use of particulates and phospholipids activate the contact group Factors XIII, prekallikrein, and high molecular weight kininogen. PTT evaluates intrinsic and common pathways.

The point of care coagulation analysis instrument (SCA2000) is available for in-house use and can rapidly determine both the PT and PTT on small amounts (50ul) of citrated whole blood. Until the past decade when this
analyzer became available, the only bench top tests available for clinical use included the ACT tube test and the PIVKA test.

- **PIVKA**: The PIVKA test measures the “Proteins Induced by Vitamin K Antagonism/Absence” in the circulation. Elevated levels of inactive or non-functional precursors of Vitamin K1 in conjunction with a suspicious history and clinical signs of a clotting dysfunction are consistent with Vitamin K1 deficiency. The PIVKA test detects any coagulation factor deficiency of the extrinsic and common pathway and is not specific for the detection of anticoagulant rodenticide poisoning.

To get a global evaluation of an animal’s hemostatic condition, often screening for a coagulopathy is done in a step-wise manner.

**Screening Tests:**

- CBC with platelet count, PCV/TS: platelet number, anemia
- ACT: general assessment of secondary hemostasis
- BMBT: to assess primary hemostasis
- PT: to assess secondary hemostasis -- a prolonged PT indicates a coagulation factor deficiency in the extrinsic (factor VII) system or common system
- PTT: to assess secondary hemostasis – a prolonged APTT indicates a coagulation factor deficiency in the intrinsic (factors XII, XI, IX, VIII) system or common system (factors X, V, II, and fibrinogen)

**Definitive Diagnostic Tests:**

- vWF : Elisa, multimeric analysis of vWf antigen, or genetic testing for von Willebrand disease.
- Specific Factor Assays: Assays of plasma for specific coagulation factors are done in a few hemostasis research laboratories.
- Fibrin degradation products (FDP’s) and D-Dimers (specific FDP’s): Assays of the products of breakdown of fibrin by plasmin is done by commercial latex agglutination kits. These tests measure the activation of the fibrinolytic system and while increased amounts are often seen in DIC and in thromboembolic diseases, they are not specific tests for these diseases. The same can be said of the presence of schistocytes (fragmented red cells): they are also often associated with, but not pathognomonic for, disseminated intravascular coagulation (DIC).
### Bleeding Disorder Diagnostic Table

The diagram below indicates test results seen in a number of diseases, with N=normal, I=increased or prolonged, and D=decreased.

<table>
<thead>
<tr>
<th></th>
<th>BMBT</th>
<th>Platelet</th>
<th>APTT</th>
<th>OSPT</th>
<th>FDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune-mediated Thrombocytopenia</td>
<td>I</td>
<td>D</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Thrombopathy</td>
<td>I</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Hemophilia (Factor VIII or IX Deficiency)</td>
<td>N</td>
<td>N</td>
<td>I</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Factor XII Deficiency</td>
<td>N</td>
<td>N</td>
<td>I</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Rodenticide Toxicity (Vitamin K antagonist)</td>
<td>N</td>
<td>N or D</td>
<td>I</td>
<td>I</td>
<td>N or I</td>
</tr>
<tr>
<td>Factor X Deficiency</td>
<td>N</td>
<td>N</td>
<td>I</td>
<td>I</td>
<td>N</td>
</tr>
<tr>
<td>Disseminated Intravascular Coagulation</td>
<td>I</td>
<td>D</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>von Willebrand's disease</td>
<td>I</td>
<td>N</td>
<td>N or I</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Table: K. Jane Wardrop, DVM, MS, DACVP. Diagnosis of Bleeding Disorders (VET-56), Western Veterinary Conference 2004, College of Veterinary Medicine, Washington State University, Pullman, WA, USA.

### Coagulopathy Culprits: “The Big Three”

I. Vascular disorders—diseases such as vasculitis can cause hemostatic defects but these are rare and shouldn’t be considered until you have assessed all the other hemostatic options.

II. Primary Hemostatic Abnormalities

- Vascular disorders—diseases such as vasculitis can cause hemostatic defects but these are rare and shouldn’t be considered until you have assessed all the other hemostatic options

- Thrombocytopenia: most common cause of defective primary hemostasis

  i. Decreased Platelet Production
     1. Chemotherapy
     2. Drugs such as estrogen
     3. Myelofibrosis
     4. Megakaryocyte hypoplasia
     5. Aplastic Anemia
     6. Rickettsia (esp. chronic ehrlichiosis)
     7. Myeloproliferative disorders
ii. Increased Platelet Destruction
   1. Consumptive Coagulopathies (i.e., DIC)
   2. Immune-mediated Thrombocytopenia (ITP)
   3. Rickettsia (esp. acute ehrlichiosis or RMSF)

- Defective Platelet Function
  i. Congenital defects (esp. vWS)
  ii. Drugs/Medications (NSAIDS esp. aspirin)
  iii. Uremia
  iv. Hepatic Diseases
  v. DIC (mediated by FDP)

III. Secondary Hemostatic Abnormalities

- Clotting Factor Deficiencies—these are the most common cause of defective secondary hemostasis
  i. Decreased Production of Clotting Factors
     1. Hereditary (i.e., Hemophilia A –Factor VIII deficiency)
     2. Deficiency of Vitamin K (anticoagulant rodenticides, choleostasis)
     3. Hepatic Failure
  ii. Increased Consumption of Clotting Factors (DIC)

- Circulating Inhibitors of Coagulation
  i. Heparin
  ii. Fibrin degradation products (produced as a end result of DIC)
  iii. Lupus anticoagulant (antibody against clotting factors –very, very rare in small animals)
### Hemostatic Tests in Clinical Practice

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Dog</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV %</td>
<td>37-55%</td>
<td>Anemias; May not be evident in first few hours</td>
</tr>
<tr>
<td>Total Protein</td>
<td>5.5-7.5 g/dl</td>
<td>Hypoproteinemias with external blood loss</td>
</tr>
<tr>
<td>Platelet estimate</td>
<td>8-15 per oil field (1/15,000ul)</td>
<td>Thrombocytopenias</td>
</tr>
<tr>
<td>Platelet count</td>
<td>150-400,000/ul</td>
<td>also schistocytes</td>
</tr>
<tr>
<td>Buccal mucosal bleeding time (BMBT)</td>
<td>&lt; 4 minutes</td>
<td>Thrombopathies</td>
</tr>
<tr>
<td>von Willebrand factor (vWF)</td>
<td>65-150%</td>
<td>von Willebrand disease</td>
</tr>
<tr>
<td>Activated clotting time (ACT)</td>
<td>&lt;110 seconds (tube)</td>
<td>Intrinsic and common coagulopathies</td>
</tr>
<tr>
<td>Partial Thromboplastin Time (PTT)</td>
<td>12-16 seconds (Lab*)</td>
<td>Intrinsic and common coagulopathies</td>
</tr>
<tr>
<td></td>
<td>54-94 seconds (SCA 2000)</td>
<td>Intrinsic and common coagulopathies</td>
</tr>
<tr>
<td>Prothrombin time (PT)</td>
<td>10-14 seconds (Lab*)</td>
<td>Extrinsic and common coagulopathy</td>
</tr>
<tr>
<td></td>
<td>12-16 seconds (SCA 2000)</td>
<td>Extrinsic coagulopathy, not specific for warfarin</td>
</tr>
<tr>
<td>Protein Induced by Vitamin K</td>
<td>&lt;25 seconds</td>
<td>Extrinsic coagulopathy, not specific for warfarin</td>
</tr>
<tr>
<td>Antagonism/Absence (PIVKA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombin time (TT)</td>
<td>10-12 seconds (Lab*)</td>
<td>Hypofibrinogenemia functional</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>100-300 mg/dl (precipitated)</td>
<td>Hypofibrinogenemia</td>
</tr>
<tr>
<td>Fibrin split products (FSP/FDP)</td>
<td>&lt;1:5 (Lab*); &lt;5ug/dl (Lab*)</td>
<td>Fibrin(-ogen) degradation</td>
</tr>
<tr>
<td>D-dimers</td>
<td>&lt;250µg/dl; negative/positive (kit)</td>
<td>Fibrin degradation</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>90-120% (Lab*)</td>
<td>Low levels with thrombosis</td>
</tr>
</tbody>
</table>

*A search for an underlying disorder should also be pursued.

* Use veterinary laboratories established reference range.

SCA 2000 instrument using fresh citrated whole blood.

Table: Urs Giger, Diplomate ACVIM & ECVIM. *Practical Approach to the Bleeding Patient*, WSAVA 2002 Congress, Veterinary Hospital, University of Pennsylvania.
**Buccal Mucosal Bleeding Time (BMBT)**

**Materials**
- Bleeding time device
- Gauze strip
- Filter paper or gauze sponges
- Timing device

**Procedure**
1. Place animal in lateral recumbency.
2. Expose mucosal surface of upper lip. Position a gauze strip around the maxilla to fold up the upper lip. Tie the strip gently, just tight enough to partially block venous return.
3. The incision site should be void of surface vessels and slightly inclined so that shed blood from the incision can flow freely toward the mouth. Place bleeding time device flush against mucosal surface, applying as little pressure as possible, and press tab to release scalpels.
4. Let stab incisions bleed freely, undisturbed, and time until bleeding stops. Excessive blood should be blotted as often as necessary so as not to have blood flow into patient's mouth. Place either filter paper or gauze sponge approximately 3-4 mm below the incision, taking care not to disturb the incision site and any clot that may be forming.
5. The end point is recorded when the edge of the filter paper/sponge does not soak up free-flowing blood. The bleeding time is the mean bleeding time for the two incisions. Normal bleeding time is less than 4 minutes.

**Normal BMBT:**
- Dog: 1-5 minutes
- Cats: 1 to 3.5 min

**Activated Clotting Time (ACT)**
This is an easy test to do in the hospital and it screens for abnormalities in the clotting cascade. Prolongation of ACT occurs with severe factor deficiency in the intrinsic and/or common clotting pathway (e.g., Hemophilia), in the presence of inhibitors (e.g., heparin, warfarin), or in cases of severe thrombocytopenia due to the lack of platelet phospholipid (mild prolongation of 10-20 seconds). The ACT is inexpensive, easily learned, quick to perform, reproducible, and provides immediate results. It is a very useful measurement of coagulation in emergency situations

**Materials**
- ACT tube containing diatomaceous earth
- 37°C electric heat block (can substitute hot water bath or hold in hand)

**Procedure**
1. Warm ACT tube in heat block to 37°C for approximately 3 minutes.
2. Perform clean venipuncture on an unthrombosed vessel. Discard the first few drops of blood to eliminate tissue thromboplastin, the tissue factor responsible for activation of the extrinsic pathway.
3. Collect approximately 2 milliliters of blood. Begin timing as soon as blood enters the tube.
4. After collection, invert the tube several times to mix with diatomaceous earth and place in heating block.
5. After thirty seconds from start of timing, gently tilt the tube and examine for clot formation. Return tube to heat block and repeat procedure every ten seconds.
6. The ACT time is the time from collection of blood in the tube to initial clot formation. In the dog, the normal is 60-110 seconds. In the cat, the normal is 50-75 seconds.

Instructions provided courtesy of: Donna Cassioli, DVM. *Bleeding Emergencies, “Food For Thought” Veterinary Technician Continuing Education Program. VCA NWVS, August 2009.*
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