INTRODUCTION

Hemostasis results through a complex interaction between platelets, blood vessel wall and coagulation factors. This system is also intimately tied to inflammatory responses so that diseases that are associated with significant inflammation will also activate the hemostatic system, for instance sepsis, infectious canine hepatitis, FIP, and IMHA. The ultimate goal of the hemostatic system is to form a clot to prevent further hemorrhage. Counterbalancing the clotting reaction is fibrinolysis and a variety of proteins meant to inactivate active clotting factors. This helps limit clotting to the area where it is needed. Hemostasis can be divided into primary hemostasis, coagulation and fibrinolysis. By knowing the clinical signs of various hemostatic problems and a few simple in house tests it is often possible to narrow down the cause of abnormal hemorrhage and be able to develop an appropriate therapeutic and diagnostic plan.

PRIMARY HEMOSTASIS

This is a result interaction between blood vessel walls and platelets and functions to seal small and large rents in vessels rapidly. Effective primary hemostasis is dependent upon platelet numbers, platelet function, vessel wall integrity and the presence of von Willebrand factor (vWF). As a result defects in any of these areas can result in problems forming an initial platelet plug. Petechia, ecchymoses, epistaxis, hyphema, pinpoint retinal hemorrhages, and hematuria are the typical clinical signs. It is rare to see extensive bruising or hemorrhage into body cavities (thorax, pericardium, abdomen, joint).

The most commonly seen problem of primary hemostasis is thrombocytopenia. This can be caused by decreased production (bone marrow disease, Ehrlichia, neoplasia, estrogen), increased use (or loss as through DIC, hemorrhage, rat poison) or increased destruction (immune-mediated thrombocytopenia, Ehrlichia).

It is also possible, though rare, to see problems with platelet function. There are congenital diseases that have been identified though they are uncommon. More common are acquired platelet function defects. The use of aspirin can result in platelet function problems. Thrombocytopenias can also be seen when abnormal proteins coat the platelets (usually a gammopathy), this is occasionally encountered with Ehrlichia or multiple myeloma. If a procedure is planned that could be associated with hemorrhage in an animal with a significantly elevated globulin level, I do recommend a buccal mucosal bleeding time (BMBT) be performed first.

Vasculitis tends to relatively rare. It can be seen with rickettsial diseases such as Rocky Mountain Spotted Fever or FIP. Generally these diseases cause DIC by having widespread endothelial damage.

Von Willebrand disease represents the most common inherited hemostatic disorder encountered. There are various forms of the disease depending upon whether all forms of vWF are reduced, only the large multimers are missing or there is no vWF at all. The disease is common in Dobermans, although it is rare to see spontaneous clinical bleeding. Usually these animals just tend to ooze more. In the severe forms of VWD as seen in Scotties and other breeds it is possible to see fatal hemorrhages. Generally the most severe forms of the disease tend to cause premature death at an early age so that it is rarely seen in practice. Genetic testing is available for some forms of the disease, though data from most of these tests have not been subjected to peer review scrutiny.

Testing for defects of primary hemostasis

First do a blood smear, it is cheap and quick and allows you to estimate platelet numbers. If there are more than 7 platelets/hpf it is unlikely that clinical bleeding is from thrombocytopenia (actually probably it is closer to 4/hpf, 1 platelet/hpf represents approximately 20,000 plts/ul, below 80,000 it is rare to see bleeding unless a concurrent problem is present). The smear must be good though and the feathered edge must checked to make sure there aren’t clumps causing the appearance of thrombocytopenia. This test should be done in bleeding patients immediately since the answer can be had in a few minutes, don’t wait till you get a count back from a machine. Do not trust a machine count that shows thrombocytopenia, a smear has to be checked to rule out clumping.

If platelet numbers are normal and a defect in primary hemostasis is still suspected a BMBT is the next logical step. Of course a history should first determine if there has been any NSAID consumption recently. A biochemical profile or total solids from a spun PCV will generally rule out a gammopathy. If a Simplate or similar device is needed to deliver a standardized cut, gauze to tie the muzzle back and filter paper to blot off blood. The muzzle is tied back with the gauze to mildly engorge the veins and expose the buccal mucosa. The Simplate device contains a spring-loaded lancet (in dogs the Simplate II has 2 lancets, for cats use the Simplate I which has one lancet only). Timing begins once the device is fired. Blood is gently blotted away at 5-second intervals, avoiding direct contact with the wound. Normal BMBT times are less than 3 to 5 minutes. This is a test that is somewhat subjective and it does require practice to get reproducible results, an individual reference range should be established for the person doing the test.

If an unexplained prolonged BMBT is found in an animal with normal platelet numbers, plasma should sent out for vWF analysis. If this comes back normal, then a platelet function defect or vasculitis may be present. Deep skin biopsy can rule out
vasculitis. Most platelet function defects require complicated testing procedures that can only be done at certain referral practices. It usually also requires the presence of the patient to run these tests (platelet aggregation, etc.).

**COAGULATION**

Coagulation occurs after a primary plug has formed and forms the permanent blood clot. It depends upon a variety of inactive proteins (coagulation factors) that interact with each other to become activated. Classically, a cascade theory has been proposed where one activated factor activates the next. Unfortunately this is only true for glass tube work. In a biological system, everything interacts with everything else, including the blood vessel wall. Coagulation is arbitrarily divided into three systems based upon laboratory evaluation, the extrinsic, intrinsic and common cascade. Clinically, coagulopathies are of more concern than disorders of primary hemostasis since hemorrhage is more profound. Bleeding into body cavities and large bruises are common.

Both inherited and acquired coagulopathies exist. Hemophilia (A and B) is uncommonly encountered as are a variety of other congenital defects. Acquired coagulopathies are much more common. Vitamin K antagonist rodenticides (cumarin, warfarin, brodifacoum, etc.) cause a significant coagulopathy to develop by interfering with Vitamin K recycling. Vitamin K is necessary for activation of clotting factors. This can be a fatal problem and is often not recognized immediately. Swelling under the chin and mediastinal widening are two findings that should raise suspicion. One interesting point to remember is that severe thrombocytopenia can occur in dogs with vitamin K rodenticide toxicity. We will also see coagulopathies in those cases where vitamin K is poorly absorbed or stored. This can occur with liver disease, bile duct obstruction and GI disease. With these diseases, the coagulopathy tends to be subclinical, however one has to be aware that they are frequently present, especially if liver biopsy is planned. Coagulopathies also are a part of DIC.

**Testing for defects of coagulation**

Many of the tests of coagulation were reserved for reference labs previously. Recently, point-of-care analyzers have been evaluated for their utility in small animals. These devices are commonly used in humans and have the advantage that only very small volumes of blood are needed to run the test and results are available immediately. With hemostatic testing on plasma samples it is vital that the tube is filled properly, over or under filling can have significant impact on the test results.

The intrinsic cascade involves factor XII down to X, and is assessed by APTT (activated partial thromboplastin time, also tests common pathway). Defects here are associated with hemophilia A and B. ACT (activated coagulation time) is an easy in house test with a similar spectrum for factors tested as the APTT. All that is required a heating block (in a pinch testing holding the tube in the axilla will also work) and an ACT tube. The ACT is less sensitive, but very practical. With the APTT factors have to be less than 30% of normal before prolongation occurs, with the ACT 5 to 10%. Hemoconcentration will prolong the APTT.

The extrinsic involves factor VII, the factor with the shortest half-life. It is important for antagonist rodenticide poisoning diagnosis and monitoring of treatment success. OSPT will decrease in 24 hours if Vitamin K antagonist is still present (OSPT also tests common pathway). PIVKA also tests this part of the coagulation cascade, though it is more sensitive. This test requires the appropriate test kit (Thrombotest, Nycomed) and a heating block.

**ACT**

The ACT is a rapid and simple to perform. A heating block aids in standardizing the test. If the axilla is used instead, each person should practice and establish their reference range. The test does require an ACT tube. The tube contains a contact activator. It is important that a clean blood draw occurs or tissue collects in the needle. This activates coagulation, resulting in an artifically decreased ACT. One method that is helpful to avoid this artifact is to first draw a red top tube and then place the ACT tube on the Vacutainer hub. The vacuum draws in the appropriate amount of blood. The tube is then incubated at 37° for 45 seconds. After this the tube is inverted every 5 seconds to monitor for development of a clot. In general normals run at less than 90 seconds. With severe thrombocytopenia the ACT will not clot. A point of care device (Medtronic HemoTec ACT analyzer) that measures ACT has been recently evaluated, it was found to be inferior to visual ACT determination.

**PIVKA**

The PIVKA test is a modified OSPT test. By diluting the plasma more than the OSPT, sensitivity is increased. The test is also sensitive to the accumulation of inactive coagulation factors that occur when vitamin K deficiency is present. This test is not specific to vitamin K antagonist rodenticides; it will be prolonged with many other coagulopathies that affect the vitamin K dependent factors. It has been shown that this test is especially useful for animals with liver disease. It will also tend to become abnormal earlier with vitamin K rodenticide poisoning than OSPT.

**Point-of care devices**
A variety of point-of-care devices have been evaluated for use in small animal practice. The data on their accuracy is however still quite limited. The SCA 2000 (Synbiotics) has been marketed to veterinarians. This device offers the option to run an ACT, APTT or OSPT. Recently the ACT II (Medtronic), Rapidpoint Coag Analyzer (Bayer Corp.) and the SCA 2000 were compared. The ACT II correlated well with standard fibrometer results. The SCA 2000 agreed well with the ACT II however correlation to standard tests was more variable. Currently the easily obtainable POC device for veterinarians is the Coag DX Analyzer by Idexx. This is similar to the SCA 2000. These machines do offer the advantage of being able to run coagulation profiles in practice. It is a consideration however to verify test results with standard testing whenever possible until it becomes clear whether these analyzers can replace reference lab tests or not.

DISSEMINATED INTRAVASCULAR COAGULATION

Disseminated intravascular coagulation (DIC) occurs when there is an overwhelming drive to clot. Because of the overwhelming activation of the clotting cascade overwhelms the anticoagulant mechanisms present in the body and widespread clot formation occurs, leading to thrombosis of vital organs with potential organ failure. Coagulation factors are consumed, which then results in a bleeding tendency. A classic DIC case should have a bleeding tendency together with thrombosis. Common findings include decreased platelet count, prolonged APTT and OSPT. BMBT should also be prolonged. These findings are not specific since they will present with a vitamin K antagonist as well. Diagnosis also relies on evidence of activated fibrinolysis.

Fibrinolysis leads to the formation of a variety of split or degradation products. The FDPs could in theory be generated in ways other than DIC, so they are not necessarily specific for DIC. There are a variety of kits available. Three kits tested (Thrombo-Wellcotest, Spli-Prest, Thromboscreen) were all shown to work reliably enough. They are based upon an antibody coating latex beads that links to FDPs. The assay is a visual agglutination test. These tests can be run in any practice. Special tubes are needed to draw the blood in, they generally are provided with the kit. Newer, more specific tests for diagnosis of DIC are being investigated, whether any of these will be suitable for in-house testing is unclear as of yet.

REFERENCES

Available upon request from the author.