HEMOPET

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EVALUATION of HEMOSTASIS

Recent introduction of Antech's enhanced Preanesthetic Profile [SA050; PreOp/CBC] has raised several issues with respect to obtaining proper anticoagulated blood samples for the coagulation PT and PTT portion of this profile.

Discussion of some commonly asked questions about the diagnosis of bleeding diseases is also found in Antech News, February 2006.

The following information is intended to clarify this situation:

Blood Collection to Ensure Proper Anticoagulation

Collection of blood samples into a dry syringe can result in activation of the initial coagulation cascade with prolongation of the results, despite clean venipuncture and good blood flow. This problem is most often encountered when the animal being bled is very small, has slow blood flow, or is difficult to restrain for the collection.

A second common problem with coagulation samples is overcitration, which also prolongs results, because the BTT is inadequately filled to achieve the correct 1 in 10 final citrate: blood ratio. The BTT should ideally be completely full, but must be at least 2/3 rds full to avoid artifactual coagulation results due to overcitration.

The preferred way to collect blood for coagulation assays is to preload the syringe with the appropriate amount of citrate anticoagulant. For example, if using the small 1.8 mL BTT, please draw all the citrate into the syringe and then collect enough blood to achieve a total of at least 1.5 - 1.8 mL of anticoagulated sample. Mix immediately and place the anticoagulated blood back into the original BTT. If using a larger BTT, one example would be to preload the syringe with 0.3 mL of citrate and draw up to at least 2 mLs (ideally 2.7 mLs) with blood.. The advantage of this technique is that the blood is anticoagulated as soon as it enters the syringe. If a clean venipuncture is not obtained, the needle should be replaced with another, and a second venipuncture made (the same syringe can be used). Immediately after collecting the required amount of blood, the syringe should be gently mixed. If incomplete collection occurs, the needle can be withdrawn and syringe mixed; and then another needle can be attached to complete the collection.

If proper blood collection is obtained but the results of the PT and/or PTT are abnormal, the risk of the patient to bleed excessively during or following an operative procedure can be further assessed by performing the bleeding time. The toenail or cuticle bleeding time is preferred because it evaluates both primary and secondary hemostasis and best simulates surgical severing of a blood vessel. The mucosal bleeding time, on the other hand, primarily assesses the platelet function of primary hemostasis, and does not evaluate secondary hemostasis and stabilization of the initial hemostatic plug.

Toenail or Cuticle Bleeding Time (TNBT)

With the animal in lateral recumbancy, either awake or anesthetized (sedatives and anesthetic agents will *not* significantly affect bleeding times), use a sharp, guillotine-type toe nail clipper to make a clean transection of the nail just into the quick (becomes reproducible with practice). Let the nail bleed freely undisturbed and time until bleeding stops.

Normal TNBTs are up to 5 ½ mins in dogs and 3½ mins in cats. A prolonged bleeding time or rebleeding after initial bleeding has stopped is considered abnormal.

Bleeding times measured by this transection technique are sensitive to defects in blood vessel contraction (vascultitis), platelet number and function, and coagulation. This test can be useful for:

- pre-surgical assessment of bleeding potential
- evaluation of response to therapy in bleeding animals
- determination of degree of bleeding risk (e.g., slight prolongation by 1-3 mins may be manageable conservatively without transfusion; moderate to severe prolongation by 3 or more mins usually requires treatment before surgery or to alleviate clinical signs; bleeding times of over 10 mins are significantly prolonged and require treatment).

Mucosal Bleeding Time (MBT)

Mucosal bleeding time is defined as the time period measured between an initial small mucosal surface incision and the moment the subsequent bleeding stops. Prolongation of bleeding time occurs in patients with: thrombocytopenia (< 80,000 platelet / ulL, von Willebrand disease, congenital platelet function defects, acquired platelet function defects occurring with uremia, liver failure, paraproteinemias, aspirin therapy (or other nonsteroidal anti-inflammatory drugs), and immune-mediated vasculitis syndromes. Normal MBT is less than 4 mins for dogs and less than 2 mins in cats (under ketamine [1 mg/kg] sedation). Abnormalities of the coagulation factor cascade, such as hemophilia or vitamin K antagonism, do *not* cause abnormal mucosal bleeding times.

To perform the mucosal bleeding time test:

Restrain the patient in lateral recumbency. It is generally not necessary to use chemical restraint, but the commonly used agents do not alter bleeding times significantly. Roll back the upper lip, securing it *lightly* with a gauze wrap to slightly engorge the mucosal vessels, and blot the area adjacent to the mucocutaneous junction to remove saliva or debris. Do not use alcohol or other vasodilating astringents.

Use a Simplate or Surgicutt bleeding time device to make a standardized incision to initiate bleeding. A spring-loaded device ensures a standardized incision. Begin timing from the instant of penetration and use absorbent paper to wick blood away from the edge of the incision every 5 - 10 secs. Do not blot or touch the wound, as this could disturb formation of the platelet plug. Stop timing when bleeding from the wound has completely ceased.

Common Misunderstandings

Diagnosis of Hemophilia

While a prolonged APTT with normal platelet count, PT, fibrinogen and d-dimer in a young male animal with significant bleeding is suggestive of hemophilia, fibrinogen levels uaually go up during bleeding eoisodes and at other times of stress. This can shorten the APTT result to within the upper end of the reference range, thereby masking the diagnosis. Further, if the patient is actively bleeding when tested, d-dimer can be elevated. Definitive diagnosis requires specific coagulation factor measurement for factor VIII:C (hemophilia A) and factor IX:C (hemophilia B).

Distinguishing Rodenticide Toxicosis from DIC

Platelet count is usually normal or only slightly lowered in rodenticide exposure cases, in comparison to moderately or very low in disseminated intravascular coagulation (DIC). In severe cases of DIC, the fibrinogen will be moderately to severely reduced, whereas it should be normal or nearly normal in the typical rodenticide case. The definitive finding in DIC is high or very high d-dimer concentration, except when fibrinogen is < 50 mg/dL, as significant amounts of d-dimer cannot form in the absence of sufficient fibrinogen substrate. A history of rodenticide or potential rodenticide exposure, or of an underlying disease process that could predispose to DIC, is a critical determinant in confirming the diagnosis. When in doubt, draw samples for coagulation profile and treat with vitamin K and blood products, if indicated.

Diagnosis of von Willebrand Disease (vWD)

Most cases of vWD in animals exhibit normal platelet counts, PT, APTT, fibrinogen and d-dimer, although the latter test may be elevated if the patient is actively bleeding at the time. Definitive diagnosis requires assaying for von Willebrand factor antigen (vWF:Ag). This molecule is synthesized and released from endothelial cells, and is an acute phase reacting protein so that levels can go up during bleeding and other times of stress.

Platelet-Associated Bleeding

Platelet counts can be normal or low-normal in cases of platelet dysfunction, because platelet function is not assessed by measuring the number of platelets. As heritable platelet dysfunction exists in animals (thrombopathia, thrombasthenia), and a variety of disease states and drugs are known to affect platelet function, the definitive diagnosis in the presence of a normal coagulation profile and normal vWF:Ag would be prolongation of the TNBT or BMT along with mucosal surface bleeding, eccymoses, and excessive bleeding with surgical procedures.

In certain breeds, notably the Cavalier King Charles Spaniel and its ancestor, the English Toy Spaniel, significantly low platelet counts (even as low as 20,000/µL) can be seen. These animals typically do *not* exhibit a bleeding tendency, unless some other hemostatic problem is present.

W. Jean Dodds, DVM; Antech News May, 2007.