Bleeding Disorders – Diagnosis & Management of Thrombocytopenias, von Willebrand disease, and Coagulopathies

Tulburări de sângerare - Diagnosticul și gestionarea trombocitopeniei, a afecțiunii von Willebrand și a coagulopatiilor

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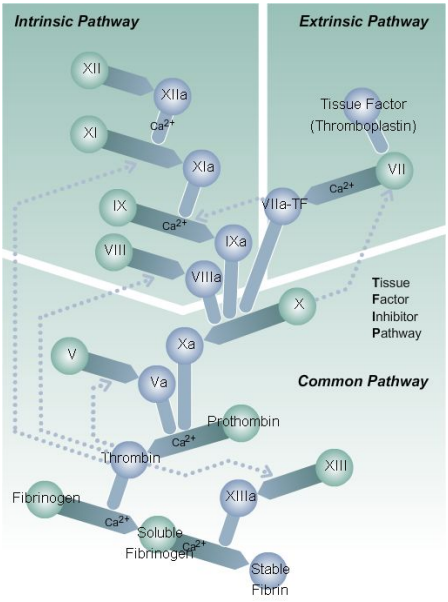
Bleeding disorders are a common presentation in dogs and less commonly in cats and may be inherited or acquired. Furthermore, thrombotic conditions are being increasingly recognized. This lecture will focus on the clinical diagnostic approach to a bleeding animal. There are several point-of-care and reference laboratory tests permitting the separation between primary and secondary hemostatic defects as well as a specific diagnosis. Particularly challenging is the diagnosis of Disseminated Intravascular Coagulation (DIC), a syndrome observed with a variety of disorders.

Bleeding diatheses are generally separated into primary and secondary hemostatic disorders and in some cases both systems are affected, such as in disseminated intravascular coagulation (DIC). Primary hemostatic disorders include not only the common thrombocytopenias but also thrombopathias, vasculopathies, and von Willebrand disease. Secondary hemostatic disorders include all coagulation factor deficiencies involved in fibrin formation and are strictly speaking the coagulopathies. Platelet and vascular problems often present with surface hemorrhage, while coagulopathies generally cause hematomas and cavity bleeds. Excessive hemorrhage at an injury or surgery site and bleeding from multiple places are suggestive of bleeding disorder, and there are a several breed predilections for specific hereditary defects.

Hemostatic tests are indicated whenever an animal is bleeding excessively, prior to surgery when an increased bleeding tendency is suspected, to monitor therapeutic interventions, and for genetic screening in certain breeds or families with a known bleeding disorder. Hemostatic abnormalities should be assessed prior to instituting therapy whenever possible or at least appropriate blood samples should be collected pretreatment. Excellent venipuncture with discarding of the first few drops of blood (to avoid platelet activation and tissue factor) and extended compression over jugular, saphenous or femoral vein is required. The **cuticle bleeding time** crudely assesses overall hemostasis, but is not standardized and painful and is, therefore, not recommended. A minimal database includes a packed cell volume and total protein evaluation, and **evaluation of a blood smear** can provide a platelet estimate and identify platelet size and clumping as well as schistocytes. The results can also provide some measure of the extent of blood loss and red blood cell transfusion requirement.



Tools for Primary Hemostatic Defects

Platelet counts can be estimated on a blood smear or specifically counted by a hematology instrument. Since 8-15 platelets (1 platelet equals 20,000/µl) are normally found per high power oil emersion microscopic field, an absence to low number of platelets suggests a severe thrombocytopenia. Various modern impedance and laser hematology instruments have the ability to count platelets and measure their mean size including platelet size distribution and platelet crit; they may have been validated, but some have difficulties in differentiating large platelets from erythrocytes (particularly in cats). Furthermore, platelets can readily be activated which results in platelet aggregation, hence, platelet counts need to be confirmed by a careful review of a blood smear including the feather edge for platelet clumps (preferably on fresh non-anticoagulated blood). Hemorrhage is generally not observed unless the platelet count is <40,000/µl (normal 150-500,000/µl) or there is also a coagulopathy like DIC.

Thrombocytopenia, a common cause of surface hemorrhage in dogs, can result from impaired thrombopoiesis, increased platelet destruction and consumption, and sequestration of platelets (splenomegaly). Reduced platelet production may be isolated or associated with an overall decreased hematopoiesis due to many drug reactions (estrogens, chemotherapeutics, azathioprine), infections (Ehrlichia spp.), and myelophthisis (leukemia, myeloma, myelofibrosis), but remains often idiopathic (immune-mediated?). Accelerated platelet destruction is commonly associated with immune-mediated thrombocytopenia (IMT, including idiopathic thrombocytopenia purura [ITP]), but enhanced platelet consumption may also be observed with neoplasia, vasculitis and disseminated intravascular coagulation (DIC). IMT can be divided into primary, also known as idiopathic thrombocytopenia purpura (ITP), and secondary forms triggered by infections (Ehrlichia, Rickettsia, and Babesia spp., Anaplasma spp., vaccines), drugs, and cancer. Anticoagulant rodenticide poisoning can also be associated with mild to moderate thrombocytopenia. However, acute and chronic blood loss is not resulting in any significant consumptive thrombocytopenia

unless there is concomitantly a vasculopathy or DIC present. Thrombocytopenia occurs rarely in cats and is generally associated with drug exposure (griseofulvin, methimazole), viral infections, or malignant diseases.

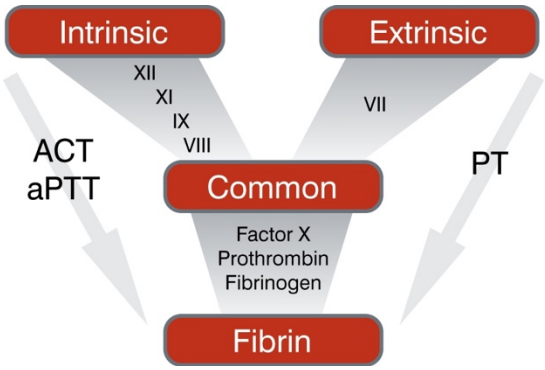
A diagnosis of thrombocytopenia is made by a platelet estimate on a blood smear or complete blood cell count, but any thrombocytopenia must be verified by a review of a blood smear. Spurious thrombocytopenia may due to instrument limitations; e.g. megaplatelets in Cavalier King Charles and few other breeds, and platelet aggregates with many illnesses and collection techniques; also Greyhounds have generally a mild thrombocytopenia. Classic signs of thrombocytopenia include petechiation, ecchymosis, epistaxis, and gastrointestinal blood loss. The most severe thrombocytopenias, seen with IMT/ITP, often cause only mild hemorrhage. Following a careful history, a search for an underlying cause is warranted to identify an infection (blood smear, serology, PCR) or cancer (also involving lymphnodes and spleen). Bone marrow examination is safe, but may rarely reveal a specific cause on initial presentation. A diagnosis of ITP is mostly based upon excluding other causes of thrombocytopenia, but platelet-associated antibodies can also be determined to support an immune mechanism for thrombocytopenia. Detection of platelet-associated antibodies further supports an immune-mediated thrombocytopenia, but this test is rarely available. Serum titer, antigen and PCR tests for tick-born (ehrlichiosis, babesiosis, leishmaniasis, Rocky mountain spotted fever) and other infectious diseases are indicated in certain countries or areas. The presence of schistocytes and thrombocytopenia suggests intravascular disseminated coagulation, where intravascular fibrin strands fragment erythrocytes. Because von Willebrand disease is such a common mild primary hemostatic defect in dogs, plasma vWF measurements by ELISA through a commercial laboratory are indicated. For breeding purposes, DNA testing is also available for some canine breeds.

Finally, in light of normal platelet count and plasma vWF values, a prolonged buccal mucosal bleeding time (BMBT) indicates a thrombopathia. Disposable devices are available that facilitate making 1-2 standard 1 mm deep mucosal incisions. The platelet function analyzer (PFA100) is a simple tool to functionally assess primary hemostasis. Electron microscopic and platelet aggregation and nucleotide studies allow further characterization of platelet dysfunctions in specialized laboratories. For a couple of hereditary thrombopathias even a DNA test is now available such as for Glanzmann thrombasthenia in Great Pyrenees and Otterhound, and thrombopathia in the Spitz, Basset, Landseer and Swiss Mountain dog, and macrothrombocytopenia in Cavalier King Charles (Auburn University).

Coagulation Tests

Whereas the whole blood clotting time test is insensitive and mostly inaccurate, there are several standardized coagulation screening tests that are useful to define coagulopathies in clinical practice. Nearly all coagulation tests assess the function of certain parts of the coagulation system in fresh whole blood or fresh (or frozen) plasma to generate fibrin in a fibrometer; recalcified citrated plasma is used and many tests are comparing a patient sample directly with a simultaneously obtained control or pooled plasma (plasma from 10 animals). Generally coagulation times, which is measuring the time to clotting (fibrin formation), are much shorter in small animals than

in humans; thus, every coagulation test needs to be run on an instrument for animals and validated for the animal species.



The intrinsic and common pathways are assessed by either the activated coagulation time (ACT) or activated partial thromboplastin time (aPTT or PTT). Factor XII of the intrinsic cascade is activated by diatomaceous earth (celite) in the ACT test and by kaolin or other contact phase substrates in the aPTT test. The extrinsic and common pathways can be assessed by the prothrombin time (PT) test. In these two assays different tissue factors (thromboplastins) are activating factor VII, which in turn will lead to fibrin formation.

Until recently the ACT tube test was the only point-of-care test available for clinical practice, whereas PTT and PT tests were performed in commercial laboratories. There are now new point-of-care coagulation instruments (e.g. IDEXX Coag DX and the Abaxis VetScan VSpro) introduced that are capable of determining without delay on small amounts (50 µl) of fresh citrated whole blood the aPTT and PT, thereby making separation of citrated plasma and shipment of frozen plasma to the laboratory for initial coagulation screening unnecessary. In practice, a reasonable and simple approach for a bleeding animal to be screened for a coagulopathy would be to measure the ACT or PTT first as either test detects all coagulopathies (except for hereditary factor VII deficiency in Beagles, Scottish Deerhounds and Alaskan Klee Kais), but the aPTT is more standardized and the ACT can only be run on fresh whole blood. If the aPTT (or ACT) is prolonged, a PT test would be indicated to differentiate between an intrinsic and common pathway defect or a combined coagulopathy involving several coagulation factors.

Although hereditary coagulopathies can be suspected based upon the pattern of coagulation test abnormalities, specific factor analyses are needed to confirm a diagnosis. A young male animal who is bleeding and has a mildly prolonged aPTT but normal PT likely has hemophilia A or B (factor VIII or IX deficiency), an X-chromosomal recessive disorder. However, factor XI deficiency is associated with the same test abnormalities and is inherited by an autosomal recessive trait (e.g. Kerry blue terriers). For several hereditary coagulopathies DNA tests are already available (http://research.vet.upenn.edu/penngen), while for others the specific plasma factor deficiency can be determined through the Comparative Hemostasis Laboratory at Cornell University. Finally, factor XII deficiency, particularly common in domestic shorthair cats, and prekallikrein deficiency causes marked aPTT prolongations but no

excessive bleeding tendency. Rodenticide poisoned animals that are bleeding or are at risk for bleeding will have severe prolongations in all of the above coagulation tests, but would have a normal thrombin time (TT). The thrombin time is independent of vitamin K-dependent coagulation factors and is a functional assay for fibrinogen to form fibrin. The protein induced by vitamin K antagonism or absence (PIVKA) test is a modified PT test and not diagnostic for rodenticide poisoning, but a toxicological investigation (product identification, blood toxicology analysis) may confirm the rodenticide poisoning. Moderate thrombocytopenia may be associated with rodenticide poisoning. All liver diseases may result in varied coagulopathies due to impaired coagulation factor synthesis and vitamin K malabsorption.

Hemostatic screening tests and groups of bleeding disorders

	Platelets	BMBT	PTT	PT	TT
Thrombocytopenia	**D**	**I**	**N**	**N**	**N**
Thrombocytopathia & vWD	**N**	**I**	**N**	**N**	**N**
Intrinsic coagulopathy	**N**	**N**	**I**	**N**	**N**
Extrinsic coagulopathy (FVII)	**N**	**N**	**N**	**I**	**N**
Combined coagulopathies (DIC, liver, rodenticide)	**D**	**I/N**	**I/N**	**I/N**	**I/N**

N = normal; I = increased (prolonged) time; D = decreased

Similarly, disseminated intravascular coagulopathies due to many different disorders is associated with variably prolonged coagulation times. More helpful to the diagnosis of DIC are the recognition of schistocytes, thrombocytopenia, low fibrinogen and antithrombin III levels, and increased D-dimers and fibrin split (degradation) products. Finally, thromboelastography (TEG or ROTEM) techniques can now be used in the emergency room, intensive care units, and referral centers to assess overall hemostasis and particularly thrombotic/fibrinolytic tendencies of citrated whole blood.

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