Article #2

Diagnosing Bleeding Disorders

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ABSTRACT:

The hemostatic system is a very complex, coordinated, and balanced interaction among endothelial cells, platelets, circulating clotting factors, fibrinolytic agents, and inhibitors of hemostasis. The purposes of the hemostatic system are to maintain vascular integrity to prevent excessive blood loss during health and injury as well as maintain adequate blood flow through the vessels to provide oxygen to tissue. Mild to fatal hemorrhage can result from defects in the hemostatic system. This article discusses normal hemostasis (i.e., primary hemostasis, secondary hemostasis, fibrinolysis, amplification and inhibitory steps), testing to evaluate the hemostatic system, and interpretation of results, with the goal of helping practitioners feel more comfortable evaluating patients with suspected bleeding disorders.

From the patient with epistaxis due to low platelets to the patient with hemothorax due to anticoagulant rodenticide poisoning, disorders of the hemostatic system can manifest in numerous ways (Figure 1). Clinicians need to be able to recognize signs of a bleeding disorder during the physical examination and to adequately evaluate the hemostatic system to make a rapid and accurate diagnosis. Hemostatic disorders can be classified as primary or secondary. Primary hemostatic disorders involve a qualitative and/or quantitative defect in platelets or vessels, whereas secondary disorders involve qualitative and/or quantitative defects in clotting factors. Primary and secondary hemostatic disorders can occur simultaneously. To understand what can go wrong with hemostasis, clinicians must understand how the normal hemostatic system works.

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NORMAL HEMOSTASIS

Endothelial Damage

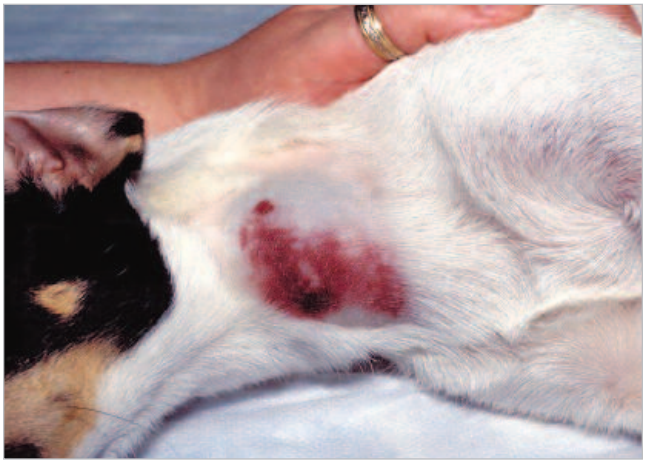
Healthy intact endothelial cells lining the vascular system are naturally antithrombotic. Negative charges on endothelial cell surface membranes repel platelets, and endothelial cells secrete substances that dilate vessels and inhibit platelet function, including prostacyclin (PGI_2), adenosine diphosphatase, and nitric oxide.[1–4] Endothelial cells separate circulating blood from thrombogenic subendothelial components such as von Willebrand factor (vWf), collagen, tissue factor (TF), and fibroblasts. vWf is also secreted into circulation and must undergo a conformational change before it can participate in platelet adhesion. Once the vascular endothelium is damaged, however, the antithrombotic capabilities of endothelial cells decrease and subendothelial components are exposed, initiating a complex and well-regulated hemostatic process.[1,4]

Primary Hemostasis

Primary hemostasis is the initial response to endothelial damage associated with either normal endothelial turnover or tissue damage and results in the formation of a platelet plug via interactions between vascular endothelium and platelets (Figure 2). When vascular endothelium is damaged, local vasoconstriction is initiated and maintained by substances secreted

Figure 1. Hemostatic defects.

Hyphema associated with defective primary hemostasis (i.e., severe immune-mediated thrombocytopenia) in a shih tzu. Hyphema can also be caused by secondary hemostatic defects.



Cervical hematoma and bruising associated with jugular venipuncture in a feist terrier with defective secondary hemostasis (i.e., anticoagulant rodenticide toxicosis). Postvenipuncture bruising can also be seen in patients with primary hemostatic defects, although hematoma formation is uncommon.

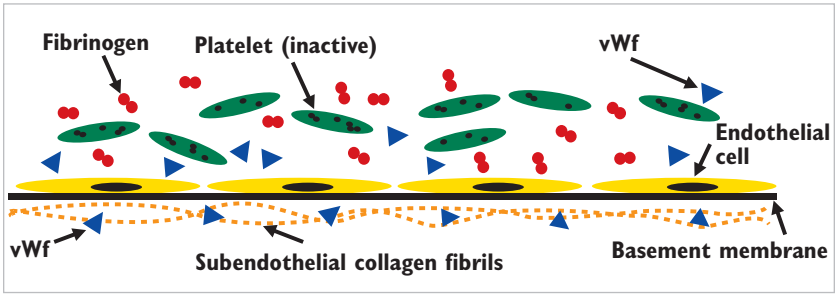
from nearby activated platelets.[2,5] Vasoconstriction decreases blood flow through the damaged endothelium. Endothelial damage also exposes subendothelial procoagulant components to the circulation. Platelets then adhere to subendothelial collagen via specific membrane receptors on the platelet—a process that initiates platelet activation. Platelets also bind to vWf—a process that promotes further platelet adherence and activation at the site of vessel injury. Activated platelets change shape to increase surface area as well as promote adherence and aggregation of other platelets via the release of the contents of platelet-dense and alpha granules.[6–8] Fibrinogen-mediated platelet-to-platelet adhesion (aggregation) follows exposure of fibrinogen receptors on the surface of activated platelets. The resultant platelet plug is composed of platelets adhered to the exposed vascular subendothelium and aggregated to each other.[1] The platelet plug provides only a temporary seal for damaged vessels and is not sufficient to sustain long-term hemostasis.

Secondary Hemostasis

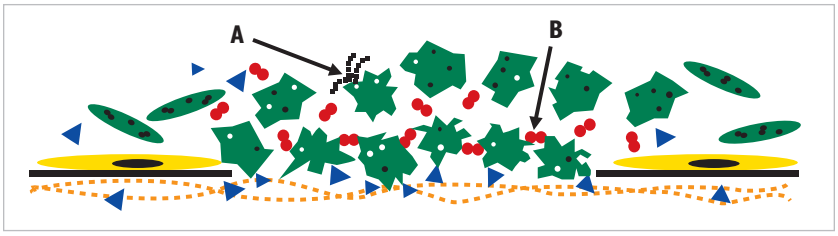
Secondary hemostasis is the process of formation of a stable fibrin clot over the already-formed platelet plug (Figure 3). Secondary hemostasis involves the sequential activation of multiple coagulation factors—a process that ultimately results in the formation of thrombin at the site of vessel damage, the central event of secondary hemostasis.[5–7] The traditional concept of the secondary hemostatic system has been of two pathways, the intrinsic and extrinsic, both activating a common pathway and leading to the formation of thrombin and, ultimately, cross-linked fibrin (Figure 3). The ultimate formation of thrombin and cross-linked fibrin is still the main endpoint of coagulation, but the distinction of separate extrinsic and intrinsic pathways leading to that endpoint is changing. The TF (extrinsic) pathway is now thought to be the main initiator of coagulation, with intrinsic factors serving to sustain the process.[1,9] The division into intrinsic and extrinsic pathways does, however, aid in interpreting coagulation

The hemostatic system involves complex interactions among primary hemostasis, secondary hemostasis, fibrinolysis, and amplification and inhibitory steps.

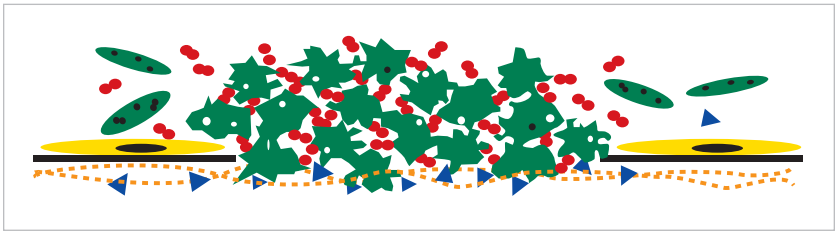
Figure 2. Outline of the events in primary hemostasis. (Reproduced with permission from Day M, Mackin A, Littlewood J [eds]: *Manual of Canine and Feline Haematology and Transfusion Medicine*. Gloucester, British Small Animal Veterinary Association, 2000.)



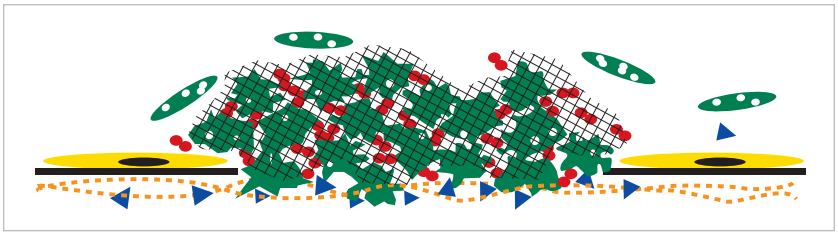
Intact endothelium and components of primary hemostasis.



Initial response of platelets to vascular damage and exposure to subendothelial collagen fibers and vWf. Activated platelets change shape, release the contents of their granules *(A)*, and form fibrinogen-mediated platelet-to-platelet bridges *(B)*.



Recruitment of platelets to the growing platelet plug in response to agonists released from the platelet granules and generation of thrombin in secondary hemostasis.



Stabilization of the platelet plug by the fibrin mesh formed in secondary hemostasis.

tests. Clotting factors, the key components of secondary hemostasis, are produced primarily by hepatocytes and are released into the circulation by the liver in inactive forms (i.e., FV, FX) that must be activated (i.e., FVa, FXa) by the clotting pathway. Vitamin K_1 is necessary for proper hepatocyte formation of functional factors FII (prothrombin), FVII, FIX, and FX.[6]

Secondary hemostasis is initiated by vascular endothelial damage—the same event that initiates primary hemostasis. Subendothelial TF is exposed to circulating blood and combines with a small amount of circulating factor FVIIa, forming the TF-VIIa complex.[1,6,10] The TF-FVIIa complex is the driving force for further activation of coagulation factors and is the classically taught "extrinsic pathway." The TF-FVIIa complex directly activates factor X. Activated FXa and FVa (FV is activated to FVa by thrombin) combine with ionized calcium on the surface of activated platelets (prothrombinase complex) to initiate the conversion of prothrombin to thrombin. The platelet surface provides the necessary phospholipids for coagulation to proceed.[10] Thrombin then converts fibrinogen to soluble fibrin monomers, which are cross-linked into an insoluble mesh via the action of factor FXIIIa—a process that is also activated by thrombin. The process of the prothrombinase complex converting prothrombin to thrombin, with ultimate formation of cross-linked fibrin, is known as *the common pathway*.[1,7]

The components of the classic intrinsic pathway are contact factors XIIa, prekallikrein, bradykinin, and high molecular weight kininogen. The contact factors are not a relevant source of thrombin generation in vivo but do serve to activate FXI and cause coagulation in vitro; therefore, they are part of laboratory coagulation testing. In live animals, instead of being activated by these contact factors, FXI is activated by thrombin generated by the TF-VIIa complex. The classic extrinsic pathway (via the TF-FVIIa complex) is therefore the main initiator of coagulation, and the classic intrinsic pathway serves as a sustainer of coagulation. Factor FXIa activates factor FIX, which combines with ionized calcium and FVIIIa (FVIII is activated to FVIIIa by thrombin) on the surface of activated platelets

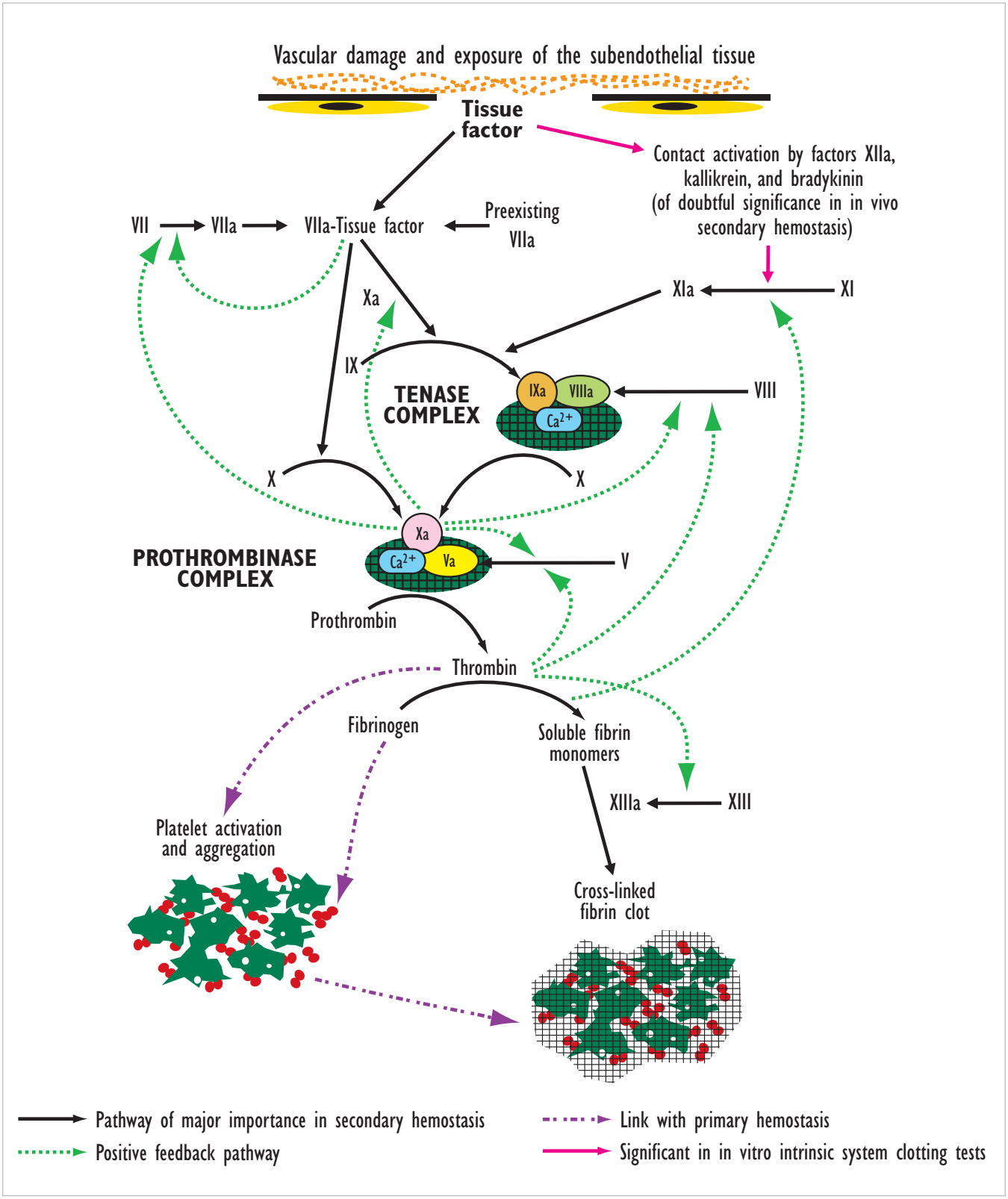


Figure 3. Outline of the events in secondary hemostasis. (Ca^{2+} = calcium) (Reproduced with permission from Day M, Mackin A, Littlewood J [eds]: *Manual of Canine and Feline Haematology and Transfusion Medicine*. Gloucester, British Small Animal Veterinary Association, 2000.)

(intrinsic tenase complex) to activate factor FX.[10] Factor Xa then forms the prothrombinase complex as already described, with ultimate formation of cross-linked fibrin. FIX can also be directly activated by TF-FVIIa (extrinsic tenase complex), which then proceeds as already described into the common pathway.

Amplification of Coagulation

The hemostatic system has numerous amplification steps that are mediated by a number of different substances (Figure 3). Thrombin is the major factor responsible for amplifying hemostasis. Thrombin maintains primary hemostasis by promoting further platelet aggregation and activation. Thrombin also sustains and amplifies secondary hemostasis by converting circulating fibrinogen to fibrin monomers and by activating factors FXI, FVIII, FV, and FXIII.[1,6] These factors then continue their normal role in coagulation, sustaining the process. Autoactivation of FVII by the TF-FVIIa complex is another important step of amplification. The presence of both ionized calcium and phospholipids is necessary for many steps in the coagulation process. Ionized calcium is normally present in adequate amounts in the circulation, whereas phospholipids are provided by platelet membranes within the primary platelet plug.[7] Platelet phospholipids serve to localize secondary hemostasis to the site of vessel injury.

Inhibition of Coagulation

As with all body systems, there is homeostatic regulation of the clotting system to maintain a balance between coagulation and anticoagulation. Various inhibitors of coagulation serve an important role in preventing excessive and uncontrolled clot formation. The most abundant and important inhibitor of clotting is antithrombin (previously known as *antithrombin III*). Antithrombin is produced by the liver and inhibits thrombin, FIXa, FXa, and FXIa.[4,7] Combination with heparan sulfate on the surface of endothelial cells significantly improves the inhibitory activity of antithrombin. This helps control coagulation at the edges of damaged vascular endothelium and thereby localize clot formation to the site of injury.[4] Other inhibitors of coagulation include TF pathway inhibitor (TFPI) and proteins C and S. In the presence of ionized calcium, TFPI complexes with FXa and is then able to complex with TF-FVIIa, thus inhibiting each of these factors. This occurs at the site of vessel injury because TFPI is bound to endothelial cells and released by activated platelets.[6] Proteins C and S are produced by the liver and, like the procoagulant clotting factors II, VII, IX, and X, are vitamin K_1 dependent. Thrombin, when bound by thrombomodulin on the surface of the endothelial cell, loses its procoagulant properties and instead activates protein C. Activated protein C (APC) binds with its cofactor, protein S, and this complex inactivates FV and FVIII.[1,6,7]

Fibrinolysis

Fibrinolysis is the process of dissolution of the fibrin clot and is therefore a prohemorrhagic event. Fibrinolysis is necessary to repair damaged vascular endothelium and restore normal blood flow through injured blood vessels.[7] Fibrinolysis is mediated by plasmin—a protein produced by the liver and released into plasma as an inactive precursor (plasminogen).[7,11] Tissue plasminogen activator (tPA) is the main plasminogen activator responsible for converting circulating plasminogen into plasmin. Endothelial cells produce and release tPA, which then binds to fibrin clots, localizing it to the site of the clot formation.[1] tPA is then able to bind and convert plasminogen to plasmin. Plasmin degrades soluble fibrin, fibrinogen, and cross-linked fibrin into fibrin (or fibrinogen) degradation products (FDPs). FDPs have antihemostatic properties and can inhibit the function of both platelets and various clotting factors. D-Dimers are produced along with FDPs when cross-linked fibrin is degraded.[9,11,12] The contact factors from the classic intrinsic pathway play a role in stimulating the conversion of plasminogen to plasmin and are, therefore, mediators of fibrinolysis.[4]

Secondary hemostasis, the process that generates a fibrin clot, is initiated by the extrinsic pathway, sustained by the intrinsic pathway, and amplified by thrombin generated by the common pathway.

Inhibition of Fibrinolysis

Fibrinolysis is inhibited through inhibition of plasmin or tPA. The main inhibitors of free plasmin are α_2-antiplasmin and α_2-macroglobulin.[6,13] Antiplasmin also interferes with the binding of plasminogen to fibrin. Without this binding, plasminogen cannot be converted to plasmin. tPA is primarily inhibited by plasminogen activator inhibitor–I (PAI-I), which is secreted by endothelial cells and platelets.[1,4,13] tPA and PAI-I circulate bound together, preventing tPA from causing systemic fibrinolysis. After this complex has bound itself to the fibrin meshwork, PAI-I is released back into circulation.[13]

Although the various steps in hemostasis are usually described sequentially for simplicity and ease of understanding, in reality, primary and secondary hemostasis, fibrinolysis, and the various events that amplify or inhibit these processes all occur simultaneously at the site of vessel injury. Hemostasis is a very complex process, and understanding the intricacies of the hemostatic system is probably still in its infancy.

Evaluation of activated clotting time or activated partial thromboplastin and prothrombin times, platelet numbers, and buccal mucosa bleeding time can provide a rapid and practical in-house assessment of a patient's hemostatic abilities.

HEMOSTATIC TESTING

Blood Collection

It is very important for proper test results that the sample be drawn atraumatically. With repeated attempts to penetrate the vessel lumen, the coagulation system becomes increasingly stimulated (i.e., release of TF, activation and consumption of platelets, clotting factors and anticoagulants), making proper interpretation of the test results difficult. The goal should be to perform a clean venipuncture and produce a freely flowing blood sample directly into a syringe predrawn with anticoagulant. The standard anticoagulant is 3.2% or 3.8% sodium citrate (clinicians should use the concentration recommended by their laboratory), and the proper ratio is one part citrate to nine parts whole blood. This is produced with 0.3 ml of citrate to 2.7 ml of whole blood, 0.2 ml of citrate to 1.8 ml of whole blood, or 0.1 ml of citrate to 0.9 ml of whole blood. Because many clinics do not have bottles of citrate solution available, whole blood can be collected via a clean syringe (without anticoagulant) and then placed into a commercially available collection tube (taking care to add exactly the volume of blood specified on the tube) containing a fixed amount of citrate. Coagulation test results can be altered if the citrate:blood ratio is not correct.[6] Use of heparin or EDTA is not an acceptable alternative. If not conducting the coagulation testing in-house immediately after sample collection, clinicians should consult their laboratory regarding sample handling.

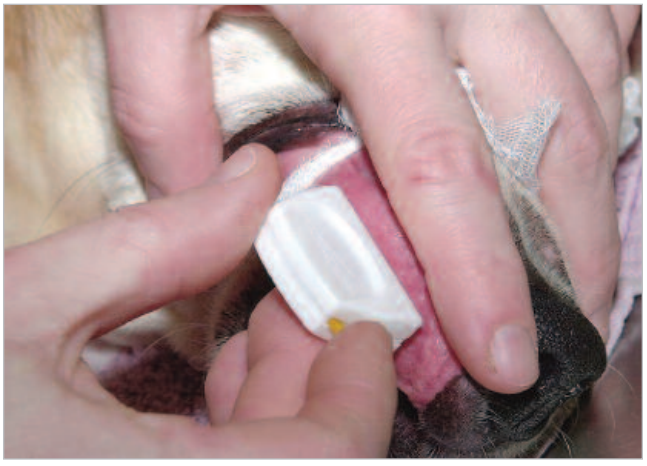
Tests for Primary Hemostasis

Platelet Counts

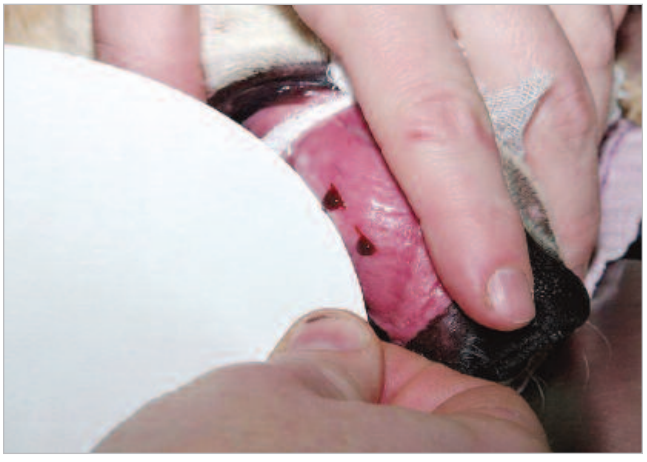
Platelet counts can be either quantitative or semiquantitative. Various automated cell counters can be used to obtain specific platelet numbers, and because thousands of cells can be counted rapidly, automated analyzer platelet counts are usually more accurate than manual platelet estimation methods, particularly in dogs. However, there is a margin of error with these counts, so two counts may vary by a few thousand platelets per microliter. If an automated analyzer is not available or an automated count is suspected to be erroneous (a common problem in cats resulting from clumping), a platelet estimate can easily be conducted in the clinic by examining a freshly made, air-dried blood smear stained with a standard hematologic stain. First, the feathered edge of the smear should be examined under low power to ensure that no platelet clumping is present. An accurate platelet estimate is not possible if clumping is seen, although the presence of numerous platelet clumps usually indicates adequate platelet numbers. Second, if clumping is not present, the smear should be evaluated under high power (oil immersion). Each platelet seen per high-power (1,000×) monolayer field is equivalent to approximately 15,000 to 20,000 platelets/µl.[14,15] Spontaneous bleeding typically does not occur if the total platelet count is over 35,000 to 50,000 platelets/µl.[14,15] Examination of a blood smear also

Figure 4. Obtaining the buccal mucosa bleeding time.

The lip is gently tied back to hold it in place and provide mild venous congestion. Sedation may be needed.



A standard incision is made in the inside of the upper lip at the level of the maxillary canine tooth.



Filter paper is used to blot the blood from the incision without disrupting the clot. The buccal mucosa bleeding time is the time from creating the incision until bleeding stops.

allows recognition of various platelet morphologic characteristics, such as the large megathrombocytes (i.e., "shift" or "stress" platelets) often seen in situations of increased thrombopoiesis.

Buccal Mucosa Bleeding Time

The buccal mucosa bleeding time (BMBT) is an in vivo test used to evaluate primary hemostasis (Figure 4). In the presence of adequate platelet numbers, the BMBT primarily serves as a test of platelet and vessel wall function.[5] The patient's upper lip should be everted and held in place with a gauze tie around the entire muzzle (in dogs) or maxilla (in cats). The gauze tie also causes mild venous congestion of the lip. A commercial spring-loaded bleeding time device should then be used to make a standardized incision in the buccal mucosa above the maxillary canine tooth.[16,17] Arguably, a scalpel blade should not be used if a specialized device is not available because test results may be unreliable resulting from too great a variability in the depth of the incision. Filter or blotting paper should be used to carefully remove excess blood without touching the incision and disrupting the hemostatic plug. The BMBT is the time from incision to initial cessation of bleeding. The BMBT can usually be obtained in awake or sedated dogs but typically requires sedation and, sometimes, full anesthesia in cats.[8,18] Normal BMBT is 1.7 to 4.2 minutes in healthy dogs[1,12] and 1 to 2.4 minutes in healthy cats.[1,19]

von Willebrand Factor

vWf plays a vital role in initial platelet adherence at the site of vascular injury, and without this factor, primary hemostasis is defective. Because von Willebrand disease (i.e., quantitative or qualitative defects in vWf) is by far the most common congenital primary hemostatic defect, testing for vWf is often indicated in young animals with suspected bleeding disorders.[20,21] Various tests, including ELISAs, multimeric analysis, vWf–collagen binding assays, and ristocetin cofactor activity, are available at specialized laboratories for evaluating both the quantity and functional integrity of vWf in the plasma. In commonly affected breeds, DNA testing for von Willebrand disease is often also available.[22] The laboratory conducting the analysis should be contacted for specific handling instructions before sample submission.

Specialized Platelet Function Tests

Specialized platelet function tests such as platelet aggregometry can be used if there are clinical signs of a

primary hemostatic disorder or a prolonged BMBT but platelet counts and vWf tests are normal. Platelet function tests are not commonly conducted and are usually limited to teaching institutions or specialized laboratories. A history of drugs that can interfere with platelet function (e.g., NSAIDs) should be excluded before patients are subjected to specialized platelet function testing.[2] Molecular assays for diseases such as Glanzmann's thrombasthenia in otter hounds and Great Pyrenees are available at the Department of Pathobiology at Auburn University.

Tests for Secondary Hemostasis

Activated Clotting Time

The activated clotting time (ACT) is a simple and inexpensive test used to evaluate the intrinsic and common pathways. The ACT is conducted by adding whole blood to a specialized tube containing a contact activator such as diatomaceous earth. The contact activator activates factor XII, with subsequent activation of the remainder of the intrinsic and common pathways, resulting in clot formation. To conduct the test, 2 ml of whole blood drawn via atraumatic venipuncture should be added into an ACT tube. The tube should be gently mixed and then placed into a heating block with a constant temperature of 98.6°F (37°C) for 60 seconds. The ACT tube should then be removed from the heating block and gently rotated and observed for clot formation. If a clot cannot be seen, the tube should be replaced in the heating block for 10 seconds. It should be removed again, gently rotated, and observed for clot formation. This process should be repeated until a clot is seen[18,23] (Figure 5). If a heating block is not available, an alternative method is to use a Styrofoam cup (or other insulating device) to hold water that has been heated to 98.6°F (37°C; measured with a thermometer). Because maintaining an accurate and steady temperature is vital to the accuracy of the test and for proper interpretation, holding the tube close to the body (axilla) may cause too much variation in temperature. The reported normal ACT is 60 to 110 seconds in dogs and 50 to 75 seconds in cats.[18] However, clinicians should determine their own normal range based on sample handling in their practice.

The ACT is a relatively insensitive test that detects only severe hemostatic deficits. Until about 90% of a factor's activity is lost, the ACT typically remains normal. Severe thrombocytopenia (i.e., less than 10,000 platelets/µl) can cause prolongations in the ACT due to a lack of platelet phospholipids necessary for assembly of coagulation factor complexes.[23]

Figure 5. Activated clotting time. A specialized ACT tube is initially kept warm at 98.6°F (37°C) for 60 seconds after adding freshly collected whole blood. The tube is then rotated every 10 seconds until a clot forms.

Activated Partial Thromboplastin Time

The activated partial thromboplastin time (aPTT) is used for the same purposes as the ACT, which is to evaluate the intrinsic and common pathways. Phospholipid, a surface activator, and calcium are added to a citrated plasma sample to trigger the intrinsic pathway, and the aPTT is the time from addition of these factors until clot formation. Typically, samples for aPTT analysis have been collected into a citrated (blue top) tube and submitted to a reference laboratory for analysis. However, the aPTT can also be easily conducted in house with a point-of-care analyzer (SCA2000 Veterinary Coagulation Analyzer, Synbiotics Corporation, San Diego, CA; Figure 6). The accepted aPTT normal range varies with the methodology and laboratory (i.e., the values from the SCA2000 may be very different than those obtained from a veterinary laboratory). The aPTT is more sensitive than the ACT, with prolongation of the aPTT detected after loss of approximately 65% of coagulation factor activity. Unlike the ACT, the aPTT is not affected by low platelet numbers.[23]

Both the ACT and aPTT are prolonged in patients with deficiencies of factors needed to trigger the intrinsic pathway following in vitro contact activation, such as factors VIII, IX, XI, and XII. The ACT may be prolonged if any of these factors falls to less than 10% of normal, and the aPTT may be prolonged if any of these

Figure 6. The SCA2000 Veterinary Coagulation Analyzer is used for point-of-care testing of aPTT and PT. (Courtesy of Synbiotics Corporation, San Diego, CA)

factors falls to less than 35% of normal. Any of these factor deficiencies, other than factor XII, can cause clinical bleeding problems. Because the intrinsic pathway is triggered in vivo via activation of factor XI by thrombin (generated via TF-VIIa complex pathway), factor XII is not essential for normal hemostasis. Despite the presence of a very prolonged ACT and aPTT, affected patients are not predisposed to clinical bleeding.

Prothrombin Time

The prothrombin time (PT), also commonly known as the *one-stage prothrombin time*, is used to evaluate the extrinsic and common pathways. TF embedded in phospholipid membranes and calcium can be added to a citrated plasma sample to trigger the extrinsic pathway, and the PT is the time from addition of these factors until clot formation. Sample collection and handling methodology is identical to that used in measuring the aPTT, and like the aPTT, the PT can be either sent to a reference laboratory or tested with a point-of-care analyzer. Because the aPTT evaluates the intrinsic pathway and the PT evaluates the extrinsic pathway, both the aPTT and PT are typically conducted simultaneously to provide maximum information regarding the clotting pathway. Prolongation of the PT, as with the aPTT, occurs if approximately 65% of factor activity is lost. The PT is similarly not affected by low platelet numbers.[23]

Because the eventual endpoint of the ACT, aPTT, and PT is formation of a fibrin clot via the common pathway, severe deficiencies of any of the factors in the common pathway cause prolongation of all these tests.

Tests for Fibrinogen and the Fibrinolytic System

Thrombin Time

The thrombin time evaluates conversion of fibrinogen to fibrin. Thrombin is added to a citrated plasma sample, and the thrombin time is the time from addition of thrombin until clot formation. Prolongation of the thrombin time (reference ranges vary with the laboratory conducting the test) suggests absolute deficiency of fibrinogen, dysfibrinogenemia, or inhibition of thrombin by substances such as FDPs or heparin (via increasing activity of antithrombin).[6]

Fibrinogen

Fibrinogen levels can be estimated with several methods. One common quantitative assessment method, heat precipitation, can be inaccurate when fibrinogen levels are low. Qualitative assessment methods of determining functional levels of fibrinogen, such as the thrombin time, typically involve adding thrombin to a citrated plasma sample and evaluating clot formation. Low fibrinogen levels can be associated with conditions causing either decreased fibrinogen production (inherited deficiencies or liver failure) or increased fibrinogen consumption (i.e., disseminated intravascular coagulation [DIC]).

Fibrin Degradation Products

Plasmin dissolves circulating fibrinogen, soluble fibrin monomers, or cross-linked fibrin within a blood clot.[11] When this happens, FDPs (also known as *fibrin split products*) are produced. Another product of dissolution of cross-linked fibrin is D-dimer. FDPs, although generally thought of as indicating active fibrinolysis, actually only indicate plasmin activation because they can be created by fibrinogenolysis without a clot having been formed. D-Dimers indicate active thrombosis and fibrinolysis because cross-linked fibrin degradation is necessary to produce D-dimers.[24]

FDPs tend to be elevated in conditions of excessive clot formation and subsequent fibrinolysis, and measurement of FDPs is often used as a marker for detecting prothrombotic and fibrinolytic conditions such as DIC and thromboembolic disease. Blood for measuring FDPs must typically be collected into a specialized tube containing thrombin and soybean trypsin inhibitor. D-Dimers can also be evaluated as a more specific marker of fibrinolysis. D-Dimer has the additional advantage of being stable in standard citrated plasma (blue-top) tubes.[11] In most veterinary laboratories, both

FDPs and D-dimers are measured by semiquantitative latex agglutination methodology.

EVALUATING THE COAGULATION SYSTEM

Patients with hemostatic disorders typically present with signs associated with excessive or unexplained spontaneous hemorrhage. Clinical signs often associated with primary hemostatic problems include surface bleeding such as petechiae (i.e., pinpoint hemorrhages) and ecchymosis, bleeding from mucous membranes, gastrointestinal bleeding, epistaxis, intraocular and periocular hemorrhage, bleeding from multiple sites, surgical bleeding, and prolonged bleeding from lacerations and incisions. Clinical signs generally associated with secondary hemostatic problems are typically localized to only a few sites, including cavity bleeding such as hematomas, hemothorax, hemoabdomen, hemopericardium and hemomediastinum; bleeding into joints or muscles; and "rebleeding" after initial clot formation.[1,23,25,26] Patients often present with clinical signs that could be caused by primary or secondary defects (Figure 1). On occasion, patients with hemostatic disorders present for evaluation before excessive bleeding is clinically apparent. Testing of hemostasis is indicated in most patients with signs of excessive or unexplained bleeding and also in patients with a history that strongly suggests a potential hemostatic defect (e.g., recent exposure to anticoagulant rodenticide), even if hemorrhage is not clinically obvious.

Although solid knowledge of the available tests for hemostasis is essential to make a diagnosis, it is also very important to apply the tests in a logical progression. In stable patients, hemostatic testing can be conducted in a sequential fashion and results interpreted before further tests are requested. However, it is often advisable to conduct a small initial panel of screening tests, particularly in patients presenting as emergencies, and to then interpret the collective results of this "hemostatic profile." The decision of whether to start with evaluation of primary hemostasis, secondary hemostasis, or both is aided by a good physical examination and history. A minimum database for screening a patient with a suspected hemostatic disorder should include a complete blood cell count (including a platelet count), a BMBT, and either an ACT or both an aPTT and a PT (Figure 7).

In patients with suspected primary hemostatic disorders, platelet numbers should first be evaluated. If the platelet count is greater than 35,000 to 50,000 platelets/µl (i.e., mild to moderate thrombocytopenia), thrombocytopenia is likely not the cause of bleeding. A BMBT should be conducted to evaluate platelet function. If the BMBT is normal, primary hemostatic defects are very unlikely to be the cause of bleeding, and secondary hemostasis should be evaluated. If the BMBT is prolonged, a platelet function problem exists and blood should be submitted for vWf testing. If vWf measurements are normal and a primary hemostatic deficit is still suspected, specific platelet function tests should be considered. Platelet function tests should be strongly considered in breeds that have been shown to have intrinsic platelet defects (e.g., Persian cats, spitz, otter hounds, basset hounds, cocker spaniels, landseers, Great Pyrenees[2,4]; see box on page 839).

If the platelet count is less than 35,000 to 50,000 platelets/µl, marked thrombocytopenia of this magnitude is likely to be the cause of bleeding, and causes of primary thrombocytopenia should be considered. The BMBT is not indicated in patients with marked thrombocytopenia because inadequate platelet numbers can lead to prolonged bleeding times even in the presence of adequate platelet function. Secondary hemostasis should still be evaluated (via an ACT or both an aPTT and a PT) because another hemostatic defect could also be present. A consumptive coagulopathy such as DIC should be strongly considered if the ACT or aPTT and PT are prolonged in the presence of thrombocytopenia, especially if there are clinical signs consistent with DIC or a confirmed disease process that is known to cause DIC.[27] FDP and D-dimer testing can aid in diagnosing DIC. Rodenticide poisoning should also be considered because anticoagulant rodenticides can both prolong clotting times and (for unexplained reasons) cause thrombocytopenia.[28] Isolated causes of marked thrombocytopenia

Immune-mediated thrombocytopenia is the most common primary hemostatic defect, whereas anticoagulant rodenticide poisoning is the most common secondary hemostatic defect.

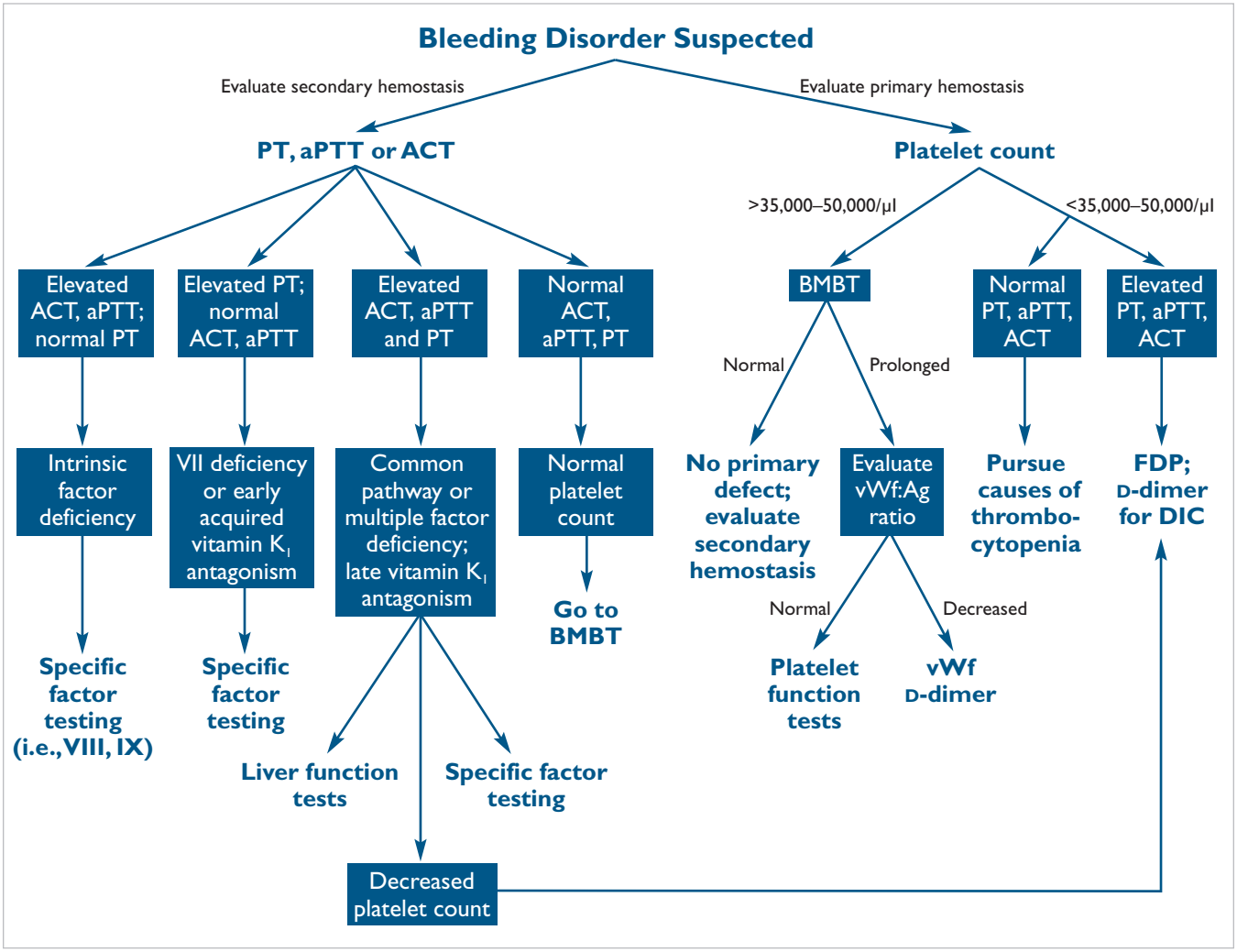


Figure 7. Flow chart summarizing the use of screening tests of primary and secondary hemostasis in the investigation of a bleeding diathesis. (*Ag* = antigen)

should be diagnostically pursued if the ACT or both the aPTT and PT are normal (see box on page 839).

In patients with suspected secondary hemostatic disorders, the ACT or, preferably, the aPTT and PT should be evaluated first. In the presence of an adequate platelet count, a prolonged aPTT or ACT and normal PT suggest a specific defect in the intrinsic pathway that, in many instances, is due to a congenital clotting factor deficiency (see box on page 842). Deficiencies of the intrinsic pathway include, particularly in male dogs, factor VIII deficiency (hemophilia A) or factor IX deficiency (hemophilia B) and, particularly in cats, factor XII deficiency (which is subclinical). In contrast, a prolonged PT and normal aPTT or ACT suggest a specific defect in the extrinsic pathway. Most commonly, specific extrinsic pathway defects occur with early anticoagulant rodenticide poisoning because factor VII, in the extrinsic pathway, is the vitamin K–dependent clotting factor with the shortest circulating half-life and is therefore the factor that tends to be depleted first as toxicosis develops. Congenital factor VII deficiency should also be considered in patients with specific extrinsic pathway defects. Prolongation of both the aPTT (or ACT) and PT suggests either an isolated congenital deficiency of a factor in the common pathway or, more commonly, a deficiency or inhibition of multiple factors in the intrinsic, extrinsic, and/or common pathways. Common acquired causes of multiple factor deficiencies include advanced anticoagu-

(text continues on p. 842)

Causes of Primary Hemostatic Disorders[2,20,22,23,29,30]

Thrombocytopenia

Decreased Production

- Immune-mediated megakaryocyte aplasia

- Drug-induced
  - — Estrogen
  - — Antibiotics
    - Chloramphenicol
    - Trimethoprim–sulfonamide
  - — Cytotoxic drugs
    - Cyclophosphamide
    - Doxorubicin
    - Azathioprine
    - Chlorambucil
    - Cytosine arabinoside
    - Methotrexate
    - Dacarbazine
  - — Methimazole
  - — Thiazide diuretics
  - — Griseofulvin (especially in FIV-positive cats)
  - — Albendazole

- Infection
  - — Chronic rickettsial disease
  - — Cyclic thrombocytopenia (*Ehrlichia platys*)
  - — Systemic mycosis
  - — Canine parvovirus
  - — Canine distemper virus
  - — FeLV
  - — FIV
  - — FIP
  - — Cytauxzoonosis
  - — Sepsis

- Neoplasia
  - — Myeloproliferative disease
  - — Lymphoproliferative disease
  - — Metastatic disease
  - — Estrogen-secreting tumor
  - — Inherited
  - — Canine cyclic hematopoiesis (gray collie)

- Other causes
  - — Myelofibrosis
  - — Idiopathic bone marrow aplasia
  - — Radiation therapy

Increased Destruction/Consumption

- Immune-mediated
  - — Primary/autoimmune
  - — Secondary
    - Systemic lupus erythematosus
    - Drug-induced
    - Infection
      - Rickettsial
      - Fungal
      - Bacterial
      - Viral (FeLV, FIV)
      - Protozoal
      - Dirofilariasis
      - *Babesia canis*
    - Neoplasia

- Nonimmune-mediated
  - — Drug-induced
  - — Ehrlichiosis
  - — Rocky Mountain spotted fever
  - — Dirofilariasis
  - — DIC
  - — Microangiopathies (hemangiosarcoma)
  - — Vasculitis
    - Systemic lupus erythematosus
    - *Ehrlichia canis*
    - *E. platys*
    - *Rickettsia rickettsii*
    - FIP
    - Canine adenovirus type 1
  - — Hepatic disease
  - — Heparin-induced
  - — Profound acute hemorrhage
  - — Hemolytic uremic syndrome
  - — Anticoagulant rodenticide
  - — Snake envenomation

- Sequestration
  - — Rickettsial
  - — Fungal
  - — Systemic lupus erythematosus
  - — Splenitis
  - — Hypothermia
  - — Sepsis
  - — Splenic torsion

Thrombocytopathia

- Inherited
  - — von Willebrand disease (many breeds)
  - — Canine thrombopathia (basset hound)
  - — Glanzmann's thrombasthenia (otter hound, Great Pyrenees)
  - — Spitz dog thrombopathia
  - — Storage pool deficiency (American cocker)
  - — Chediak-Higashi syndrome (cat)
  - — Canine cyclic hematopoiesis (gray collie)

- Acquired
  - — Drug-induced NSAIDs
  - — DIC (due to FDPs)
  - — Uremia
  - — Hepatic disease
  - — Pancreatitis
  - — Myeloproliferative disorders
  - — Dysproteinemia (myeloma)
  - — Immune-mediated thrombocytopenia

Vascular Disorders

- Inherited
  - — Ehlers-Danlos syndrome

- Acquired
  - — Vasculitis
  - — Hyperadrenocorticism

(continued from p. 838)

Causes of Secondary Hemostatic Disorders[5,6,8,17,20,21,23,30]

Inherited factor deficiency

- Factor I (fibrinogen) in dogs (i.e., St. Bernard, borzoi, collie, vizsla, Bernese mountain dog, bichon frise, other mixed breeds) and cats (i.e., domestic shorthaired, domestic longhaired)
  - — Hypofibrinogenemia
  - — Dysfibrinogenemia
- Factor II in dogs (boxer, otter hound, cocker spaniel)
  - — Hypoprothrombinemia
- Factor VII in dogs (i.e., beagle, malamute, boxer, bulldog, miniature schnauzer) and cats (i.e., domestic shorthaired)
  - — Hypoconvertinemia
- Factor VIII in dogs (i.e., German shepherd [primarily], German shorthair pointer, Labrador retriever, golden retriever, mixed breeds) and cats (i.e., domestic shorthaired, domestic longhaired, Persian, Havana brown, Siamese, Himalayan)
  - — Hemophilia A
- Factor IX in dogs (i.e., Airedale, Cairn terrier, Labrador retriever, German wirehaired pointer, American cocker spaniel, many other pure and mixed breeds) and cats (i.e., domestic shorthaired, domestic longhaired, British shorthair, Siamese)
  - — Hemophilia B
- Factor X in dogs (i.e., cocker spaniel, Jack Russell terrier) and cats (i.e., domestic shorthaired)
  - — Stuart-Prower deficiency
- Factor XI in dogs (i.e., springer spaniel, weimaraner, Kerry blue terrier, Great Pyrenees)
  - — Plasma thromboplastin antecedent deficiency
- Factor XII in dogs (i.e., miniature poodle, standard poodle, shar-pei, German short-haired pointer) and cats (i.e., domestic shorthaired, domestic longhaired)
  - — Hageman factor deficiency (does not cause clinical bleeding)
- Hereditary defects in vitamin K synthetic pathways (several dog and cat breeds)
  - — Scott syndrome (German shepherd)

Acquired factor deficiency/antagonism

- Vitamin K_1 antagonism/deficiency
  - — Anticoagulant rodenticide
  - — Severe cholestasis
- Hepatic disease
- DIC
- Heparin overdose

Resource

vetgen.com
Genetic testing for von Willebrand disease

lant rodenticide toxicosis (affecting vitamin K–dependent factors II, VII, IX, and X), liver failure, and a consumptive coagulopathy such as DIC. Inhibition of multiple factors can be caused by heparin overdose or high levels of circulating FDPs associated with DIC.[5,18,23]

Clotting times (i.e., aPTT and PT) should be reevaluated to exclude laboratory errors in patients with strongly suspected secondary hemostatic disorders and adequate platelet counts in combination with normal initial aPTT and PT results. Unusual factor deficiencies (such as factor XIII deficiency) should be considered if aPTT and PT results are still normal after repeat testing. Specialized platelet function testing may also be indicated.

REFERENCES

1. McConnell MF: Overview of haemostasis, in Day M, Mackin A, Littlewood J (eds): *Manual of Canine and Feline Haematology and Transfusion Medicine*. Gloucester, British Small Animal Veterinary Association, 2000, pp 165–171.
2. Stokol T: Disorders of platelet function, in Day M, Mackin A, Littlewood J (eds): *Manual of Canine and Feline Haematology and Transfusion Medicine*. Gloucester, British Small Animal Veterinary Association, 2000, pp 196–208.
3. Couto CG: Disorders of hemostasis, in Nelson RW, Couto CG (eds): *Essentials of Small Animal Internal Medicine*. St. Louis, Mosby, 2004, pp 1185–1199.
4. Brooks M: Coagulopathies and thrombosis, in Ettinger SJ, Feldman EC (eds): *Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat*. Philadelphia, WB Saunders, 2000, pp 1829–1841.
5. Erne JB, Mann FA: Surgical hemostasis. *Compend Contin Educ Pract Vet* 25(10):732–740, 2003.
6. Kristensen AT, Edwards ML, Devey J: Potential uses of recombinant human factor VIIa in veterinary medicine. *Vet Clin North Am Small Anim Pract* 33(6):1437–1451, 2003.
7. Stokol T: Plasma D-dimer for the diagnosis of thromboembolic disorders in dogs. *Vet Clin North Am Small Anim Pract* 33(6):1419–1435, 2003.
8. Feldman BF, Madewell BR, O'Neil S: Disseminated intravascular coagulation: Antithrombin, plasminogen and coagulation abnormalities in 41 dogs. *JAVMA* 179:151–154, 1981.
9. Dodds WJ: Bleeding disorders, in Ettinger SJ, Feldman EC (eds): *Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat*. Philadelphia, WB Saunders, 1975, pp 1679–1698.
10. Couto CG: Clinical approach to the bleeding dog or cat. *Vet Med* 450–459, 1999.
11. McConnell MF: Haemostatic diagnostic techniques, in Day M, Mackin A, Littlewood J (eds): *Manual of Canine and Feline Haematology and Transfusion Medicine*. Gloucester, British Small Animal Veterinary Association, 2000, pp 175–181.
12. Jergens AE, Turrentine MA, Kraus KH, Johnson GS: Buccal mucosal bleeding time of healthy dogs and dogs in various pathological states, including thrombocytopenia, uremia and von Willebrand's disease. *AJVR* 48:1337–1342, 1987.
13. Parker MT, Collier LL, Kier AB, Johnson GS: Oral mucosal bleeding times of normal cats and cats with Chediak-Higashi syndrome or Hageman trait (factor XII deficiency). *Vet Clin Pathol* 17:9–12, 1988.

14. Carr AP, Panciera DL: von Willebrand's disease and other hereditary coagulopathies, in Bonagura JD (ed): *Kirk's Current Veterinary Therapy XIII: Small Animal Practice*. Philadelphia, WB Saunders, 2000, pp 434–438.
15. Johnstone I: Bleeding disorders in dogs: Inherited disorders. *In Pract* 24(1):2–10, 2002.
16. de Gopequi RR, Feldman BF: Platelets and von Willebrand's disease, in Ettinger SJ, Feldman EC (eds): *Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat*. Philadelphia, WB Saunders, 2000, pp 1817–1828.
17. Hackner SG: Approach to the diagnosis of bleeding disorders. *Compend Contin Educ Pract Vet* 17(3):331–349, 1995.
18. Brooks M: Hereditary bleeding disorders in dogs and cats. *Vet Med* 555–564, 1999.
19. Parker MT, Collier LL, Kier AB, Johnson GS: Oral mucosal bleeding times of normal cats and cats with Chediak-Higashi syndrome or Hageman trait (factor XII deficiency). *Vet Clin Path* 17:9–12, 1988.
20. Feldman BF, Kirby R, Caldin M: Recognition and treatment of disseminated intravascular coagulation, in Bonagura JD (ed): *Kirk's Current Veterinary Therapy XIII: Small Animal Practice*. Philadelphia, WB Saunders, 2000, pp 190–194.
21. Mackin A: Anticoagulant rodenticides, in Day M, Mackin A, Littlewood J (eds): *Manual of Canine and Feline Haematology and Transfusion Medicine*. Gloucester, British Small Animal Veterinary Association, 2000, pp 243–251.
22. Marks SL: The buccal mucosal bleeding time. *JAAHA* 36:289–290, 2000.
23. Lewis DC: Disorders of platelet number, in Day M, Mackin A, Littlewood J (eds): *Manual of Canine and Feline Haematology and Transfusion Medicine*. Gloucester, British Small Animal Veterinary Association, 2000, pp 183–195.
24. Carr AP, Johnson GS: A review of hemostatic abnormalities in dogs and cats. *JAAHA* 31:475–482, 1994.
25. McMichael M: Primary hemostasis. *J Vet Emerg Crit Care* 15(1):1–8, 2005.
26. Donahue SM, Otto CM: Thromboelastography: A tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. *J Vet Emerg Crit Care* 15(1):9–16, 2005.
27. Rossmeisl JH: Current principles and applications of D-dimer analysis in small animal practice. *Vet Med* 224–234, 2003.
28. Bateman SW, Matthews KA, Abrams-Ogg ACG: Disseminated intravascular coagulation in dogs: Review of the literature. *J Vet Emerg Crit Care* 8(1):29–45, 1998.
29. Johnstone I: Bleeding disorders in dog: Inherited disorders. *In Pract* 2–10, 2002.
30. Good LI, Manning AM: Throboembolic disease: Physiology of hemostasis and pathophysiology of thrombosis. *Compend Contin Educ Pract Vet* 25(9): 650–658, 2003.

ARTICLE #2 CE TEST

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1. Which is the initial event in primary hemostasis?
a. vasoconstriction
b. platelet plug formation
c. fibrinolysis
d. fibrin plug formation

2. Endothelial damage is the initiating event for
a. primary hemostasis.
b. secondary hemostasis.
c. none of the above
d. a and b

3. Which plays the major role in amplifying the coagulation process?
a. FVII
b. thrombin
c. fibrin
d. calcium

4. Severe thrombocytopenia produces false results in the
a. ACT.
b. aPTT.
c. PT.
d. thrombin time.

5. Which is not a typical sign of a primary hemostatic defect?
a. petechia
b. ecchymosis
c. hemothorax
d. gingival bleeding

6. The ACT and aPTT are used to evaluate
a. platelet function.
b. intrinsic and common pathways.
c. extrinsic and common pathways.
d. only the common pathway.

7. Which is not a vitamin K_1–dependent protein?
a. FII
b. protein C
c. FV
d. FVII

8. Prolongation of aPTT and PT in the presence of thrombocytopenia is most suggestive of
a. DIC.
b. hemophilia A.
c. liver failure.
d. immune-mediated thrombocytopenia.

9. A normal platelet count with a prolonged BMBT suggests
a. an intrinsic pathway defect.
b. an extrinsic pathway defect.
c. a common pathway defect.
d. a platelet function defect.

10. D-Dimers result from breakdown of
a. soluble fibrin.
b. fibrinogen.
c. cross-linked fibrin.
d. all of the above