Hemostatic Hurdles: An interactive series of challenging bleeding cases

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Keywords: primary hemostasis, platelets, thrombopathia, von Willebrand factor, coagulation, fibrinolysis

We thank Dr. Marjory Brooks (Director, Comparative Coagulation Laboratory, Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University) for graciously providing some of the cases that will be discussed and some of the proceedings content.

1. Introduction: Clinical Diagnosis of Bleeding Disorders

Initial patient evaluation is aimed at differentiating hemorrhage from damaged or diseased blood vessels from a failure of normal hemostasis (i.e. bleeding diathesis). Bleeding diatheses are broadly classified as defects of platelet plug formation (primary hemostatic defects), defects of fibrin clot formation (secondary hemostatic defects), or defects of fibrinolysis (tertiary hemostasis).

Primary hemostasis is sufficient to stop bleeding from small vessel injury. Platelets engage exposed von Willebrand Factor (vWF) on damaged endothelium via their glycoprotein (GP) Ib-IX or vWF receptors. This in turns results in inside-out signaling that activates the GPIIb/IIIa or the fibrinogen receptor. Activated platelets also secrete their granular contents like ADP and generate thromboxane A2, which activate neighboring platelets. Activated platelets then aggregate by cross-linking fibrinogen between activated fibrinogen receptors (Figure 1). Activated platelets also expose phosphatidylserine on their membranes, setting the stage for secondary hemostasis.[1]

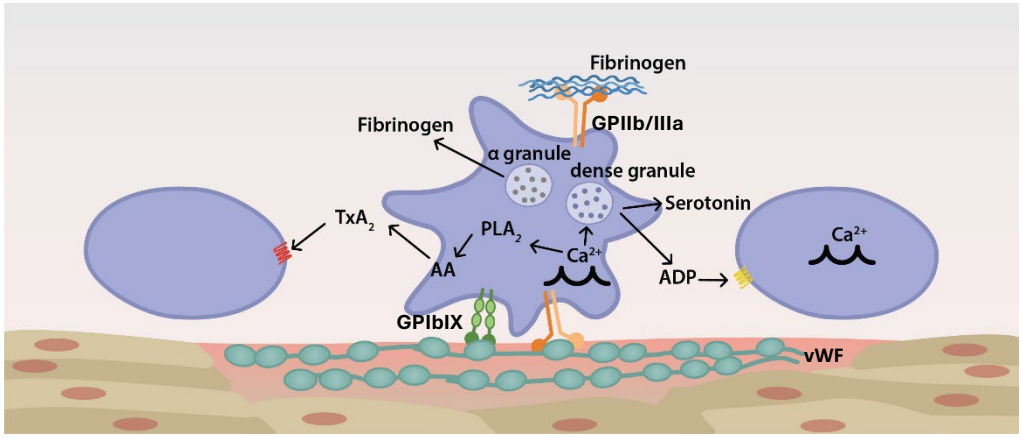

Fig 1. Primary hemostasis overview as described in text. AA = arachidonic acid, PLA_2 = phospholipase A_2, TxA_2 = thromboxane A_2, vWF = von Willebrand Factor. Figure courtesy of Andrew Kingsbury, Iowa State University.

Secondary hemostasis, or fibrin clot formation, is required to stop bleeding from large vessels. The traditional waterfall or cascade model represents secondary hemostasis accurately for laboratory testing by breaking enzymatic reactions into extrinsic (tissue factor and factor (F) VIIa), intrinsic (FXII, XI, IX, VIII), and common (FX, V, II, I, XIII) pathways. The culmination of secondary hemostasis is the generation of thrombin (FIIa) that cleaves fibrinogen (FI) to fibrin, which is then cross-linked to a stable fibrin mesh by FXIIIa.[2] However, biologically, secondary hemostasis is better depicted by the cell-based model in which all pathways are integrated into initiation, amplification, and propagation and where the process is localized to cell surfaces.[1] Initiation occurs on the surface of tissue factor bearing cells outside the vasculature that are exposed by vessel injury. Amplification and propagation occur on the surface of activated platelets.

Tertiary hemostasis describes fibrinolysis, or fibrin clot breakdown. In this phase, plasminogen binds to lysine residues on fibrin and is activated by tissue plasminogen activator (tPA) that co-binds fibrin, activating plasminogen to plasmin. Plasmin facilitates fibrin breakdown at repeated intervals into D-dimers and fibrin degradation products (FDPs). Control of fibrinolysis includes alpha-2 antiplasmin inhibition of active plasmin and plasminogen activator inhibitor-1 (PAI-1) inhibition of tPA, preventing plasminogen activation.

1.1 Clinical Bleeding History

Before delving into laboratory testing of a patient with signs of hemostatic defect, obtaining a pertinent history to help differentiate between hereditary or acquired disorders is essential. Clients must be queried about potential toxin ingestion, any medications or dietary supplements that might impact hemostasis, any previous bleeding during teething or other surgeries, and if there are any bleeding problems known in relatives or in the patient's particular breed.[3] Previous bleeding history would suggest a hereditary condition. Once a thorough history is obtained, testing can commence.

1.2 Tests of Vascular Integrity

Inspection is the primary means for identifying blood loss due vascular defects. Physical examination may be sufficient for definitive diagnosis of acute vascular injury. In other cases, ancillary diagnostics and screening tests are first performed to rule out a hemostatic defect. Vascular defects can be classified as large or small vessel disease.

a. Large vessel (arterial/venous) defects present commonly with blood-loss anemia and localized sites of hemorrhage. Tests include physical examination, endoscopy, capsule endoscopy, radiography (contrast studies), ultrasonography, CT scan, and exploratory surgery.
b. Small vessel (vasculopathic) disorders include vasculopathies such as inflammatory, toxic, and degenerative. Physical exam may reveal cutaneous erythema, macules, and bruising, and clinical signs of multi-organ involvement. Tests include serology (antibodies against pathogens and nuclear proteins),

pathogen detection, endocrine profiles (hypercortisolism, hypothyroidism), and biopsy.

1.3 Tests of Platelet Plug Formation

Platelet plug formation results from a series of interactions among vessel wall, platelets, and von Willebrand factor. Clinical signs suggestive of primary hemostatic disorders include petechiae, mucosal hemorrhage including melena, hematuria, hematemesis, epistaxis, and hematochezia, and prolonged bleeding from sites of injury. Buccal mucosal bleeding times (BMBT) will be prolonged; laboratory testing aims to differentiate quantitative and qualitative platelet disorders and vWF deficiency/dysfunction.

a. Thrombocytopenia (low platelet number): tests include platelet count or platelet estimate from blood smear. Thrombocytopenia should always be differentiated from pseudothrombocytopenia by careful blood smear evaluation. Differentials for thrombocytopenia include production defects, appropriate consumption, inappropriate consumption, splenic sequestration, and immune-mediated destruction. Thrombocytopenia is the most common acquired hemostatic defect in most species.
   Ancillary testing for thrombocytopenia might include serology, antigen and PCR tests for infectious agents (viral, arthropod-borne, & bacterial pathogens), coagulation panels, bone marrow aspiration in the case of multiple cytopenias, lymph node aspirates, thorough drug history (sulfa drugs, methimazole, chemotherapy, supplements), measurement of platelet-associated immunoglobulins, and genetic testing for macrothrombocytopenia in selected cases (see ITP Diagnosis proceedings).

b. Evaluation for thrombopathia (platelet dysfunction) can be more complicated. Starting points would be *in vivo* bleeding time (BMBT), a drug history for medications that might inhibit platelets, and a chemistry panel to look for metabolic causes of thrombopathia such as paraproteinemia. Other diagnostics could include platelet flow cytometry to assess for absence of surface glycoproteins (eg. Glanzmann's thrombasthenia) or lack of phosphatidylserine exposure with platelet activation as would occur in Scott syndrome,[4] specific platelet function tests (ex. PFA-100® closure time, whole blood and light transmission aggregometry), and mutation tests for specific breed-variants of receptors, signaling, and procoagulant defects.[5]

c. von Willebrand Disease testing could include *in vivo* bleeding time (BMBT will be prolonged), vWF concentration (vWF:Ag), vWF function (collagen-binding assays), vWF structure (Western blot), and mutation detection for specific breed-variants of type 1, 2, and 3 vWD.

1.4 Tests of Fibrin Clot Formation

Generation of a stable fibrin clot is the endpoint of secondary hemostasis. Abnormalities in secondary hemostasis result in body cavity bleeding, hemarthroses, hematomas, and prolonged or current bleeding following surgery or trauma. Testing should always begin with basic coagulation screening tests as outlined below. Functional tests include determination of *in vitro* clotting time, activities of specific procoagulant and anticoagulant factors, and tests of clot strength.

a. Coagulation screening tests: aPTT, ACT, PT, TCT, fibrinogen
    - aPTT (activated partial thromboplastin time): intrinsic and common system screening test
    - ACT (activated clotting time): intrinsic and common system screening test
    - PT (prothrombin time): extrinsic and common system screening test
    - TCT (thrombin clotting time): test of fibrinogen function and concentration
    - Fibrinogen: functional or quantitative assay of plasma fibrinogen level
b. Factor analyses: specific tests of individual clotting factor activities
c. Inhibitor assays: specific tests of antithrombin, protein C, anticoagulant drugs (i.e. unfractionated and low molecular weight heparin and anti-Xa activity, INR for coumadin monitoring) and pathologic anticoagulants (anti-factor antibody, lupus anticoagulant)
d. Viscoelastic assays (Thromboelastography, thromboelastometry, viscoelastic monitor): qualitative measure of fibrin clot formation, strength, and stability

1.5 Tests of Fibrinolysis

Hemorrhage may result from non-localized or too rapid fibrinolysis, whereas delayed or ineffective fibrinolysis may promote thrombosis and fibrotic wound healing. Fibrinolysis tests include quantitative or functional assays of fibrin clot stability, fibrinolytic enzymes, activators, inhibitors and end-products.[6]

a. Enzymes and activators can be measured, though assays are predominantly performed in research settings: t-PA (tissue plasminogen activator), plasminogen, plasmin generation.[6]
b. End products: D-dimer (terminal, cross-linked fibrin degradation products), FDP (fibrin and fibrinogen degradation products) are readily measured on coagulation panels.
c. Inhibitors: antiplasmin, PAI-1 (plasminogen activator inhibitor-1). Although these can theoretically be measured using commercially available assays, they are not routinely available laboratory tests.
d. Viscoelastic assays (thromboelastography, thromboelastometry, viscoelastic coagulation monitor): These provide qualitative measure of fibrin clot formation,

strength, and stability. Viscoelastic assays with addition of exogenous tPA have increased sensitivity for detecting altered fibrinolysis.

2 Management of Bleeding Disorders

2.1 Thrombocytopenia/thrombopathia

Thrombopathic animals should be platelet transfused when they are actively bleeding or in preparation for an invasive procedure. It remains somewhat controversial, even in human medicine, when transfusion of a thrombocytopenic patient should be performed. The authors suggest reserving platelet transfusions for thrombocytopenic patients with signs of active or refractory bleeding, especially central nervous system, pulmonary, or gastrointestinal bleeding. Predictors of disease severity are needed to identify those patients with platelet counts less than 30,000 platelets/µl that are most likely to benefit from platelet transfusions. Patients with platelets counts under 50,000/µl that require invasive procedures often benefit from platelet transfusions. Blood product choices and their pros and cons are detailed below.

Table 1. Platelet transfusion products.

Product	Platelet content	Pros	Cons
Fresh whole blood (FWB)	• 11 x 10^{10} platelets/450 ml	• No platelet loss during processing • Contains pRBC which may be ideal if anemic • Contains clotting factors if needed	• Extra volume and pRBCs if not needed • Additional antigens • Need to use blood rapidly (within 8 hours) • Time required to collect/call in donor
Packed red blood cells (pRBCs)	• Minimal platelets (~8x10^{7}/unit)	• RBC ADP release and NO scavenging may activate recipient's remaining platelets	• Does not contain a significant amount of platelets—indicated for anemia, but may help push platelets to vascular periphery where they can interact with endothelium
Platelet rich plasma (PRP)	• 8-10 x 10^{10} platelets/ unit • 1 unit/10 kg will raise platelet count maximally by 40,000/µl	• Small volume of administration • Near normal platelet function	• Requires specific equipment and staff training to prepare • 5 day shelf life • Must be gently agitated during storage • Bacterial contamination with RT storage • Reduced platelet recovery compared to FWB (80-90% platelets of FWB)
Fresh platelet concentrate (PC)	• Centrifugation preparation: 5-8 x 10^{10} platelets/PC unit • Apheresis preparation: 1.0 x 10^{11} platelets/100 ml	• Standard of care in human medicine (apheresis)	• Lack of availability • Centrifugation method results in 25% loss of platelets during processing while apheresis method requires specialized expensive equipment • 7 day shelf life* *there may be potential for prolonged cold storage of PC*

Cryopreserved platelet concentrate (CPP)	• Minimum 5 x 10^{10} platelets/100 ml	• Available immediately • Long storage time Pooled platelet product	• Reduced platelet count recovery compared with fresh platelets • Decreased platelet function • Short platelet lifespan post transfusion
Lyophilized platelets (StablePlateRX™)	• 1.5 x 10^{9}/ml	• Long storage time (up to 2 years) • Sterile • Pooled platelet product • Product consistency in platelet functionality	• Short life span after transfusion • Expensive • Currently unavailable

2.2 von Willebrand Disease

Dogs with von Willebrand Disease can be managed both prophylactically and during active bleeds with a combination of DDAVP (type I vWD only) and plasma products.

a. The ideal plasma product for vWD patients, as described below, is cryoprecipitate, though fresh frozen plasma will also be effective.
b. DDAVP is a synthetic analogue of vasopressin that works by binding to endothelial vasopressin receptors and inducing release of vWF and FVIII, leading to increased circulating plasma vWF and FVIII.[7] Although the increase in plasma vWF in dogs with type I vWD is lower (50% increase) following DDAVP administration than in humans with type I vWD (2-5X increase), 1 µg/kg DDAVP given SQ still improved buccal mucosal bleeding times and PFA-100 closure times in Dobermans with type I vWD.[7] Furthermore, the vWD type I dogs receiving DDAVP that had subsequent surgery or diagnostic procedures did not have excessive bleeding or require any blood products.[7] Two dogs that were already bleeding from another disease (tumor and inflammatory bowel disease) required plasma transfusions and still had ongoing bleeding.[7]

2.3 Secondary hemostatic disorders

Acquired and inherited coagulation disorders often respond to transfusion. While red cell transfusion is indicated if clinical signs of anemia (hypoxia) are present, plasma products can be given prophylactically and repeatedly with less risk of volume overload or red cell sensitization. Common indications for plasma components include acute rodenticide toxicity, liver biopsy/shunt correction, hemophilia, and other hereditary factor deficiencies. Patients with hereditary factor deficiencies require factor replacement when they are actively bleeding or if they require an invasive procedure.

Products:

a. Fresh frozen plasma (FFP): plasma separated from whole blood and frozen within 4 to 6 hours of collection maintains activity of coagulation factors, fibrinogen, vWF, and contains albumin and globulins. Storage at or below -

20°C maintains activity of hemostatic factors for up to 1 year. Plasma stored frozen for more than one year (FP) and less than 4 years is still useful for supplying albumin and globulin and heat stable clotting factors (II, VII, IX, and IX).

b. Cryoprecipitate: prepared by slowly thawing FFP and collecting the precipitant fraction. Cryoprecipitate contains factor VIII, vWF, and fibrinogen, in approximately 1/10 volume of the starting plasma. Cryoprecipitate is the best product for treating hemophilia A, vWD, and fibrinogen deficiencies because it is effective, lower volume, and is associated with less adverse reactions.[8]
c. Cryosupernatant (cryo-poor plasma): plasma remaining from cryoprecipitate preparation contains active clotting factors (except FVIII, fibrinogen) and albumin and globulin. Cryosupernatant is used to treat hemophilia B, hereditary factor deficiencies, vitamin K deficiency, and for replacement of albumin and globulin.

2.4 Excessive fibrinolysis

Currently available antifibrinolytic drugs are lysine analogs such as ε-aminocaproic acid (EACA) and tranexamic acid (TXA). These compounds are widely used in human and veterinary medicine to decrease fibrinolysis in diverse applications. In general, they work by reversibly binding to the lysine binding sites of plasminogen, preventing its association with the lysine residues on fibrin, thereby preventing activation of plasminogen by tPA. The most established use of these agents in veterinary medicine is in greyhound dogs. Investigating delayed postoperative bleeding in greyhounds, a group at Ohio State University hypothesized that these dogs had a hyperfibrinolytic condition and successfully minimized delayed postoperative bleeding in a population of greyhounds following limb amputation for osteosarcoma using aminocaproic acid.[9, 10] A genetic variant that may cause alpha-2 antiplasmin deficiency has been identified in Scottish deerhounds with delayed postoperative hemorrhage.[11] Other hyperfibrinolytic states have been identified in dogs including hemoperitoneum caused by a ruptured abdominal tumor, and in dogs following trauma.[12, 13] Anti-fibrinolytic agents may be indicated for use in both of these populations. In addition, canine patients at risk for hemorrhage following routine or emergent surgery may benefit from the use of antifibrinolytic agents. TXA was administered to dogs with Scott syndrome after ovariohysterectomy and castration and EACA was given to a Scott syndrome dog with a pelvic limb hematoma and use has been reported in dogs with severe ITP.[4, 14] Treatment trials will be need to determine the efficacy of antifibrinolytics to decrease transfusion requirements and prevent rebleeding in such dogs with thrombopathias and perhaps thrombocytopenic patients. Although there is a wide variety of published doses for these drugs, in dogs the authors use 10 -20 mg/kg TXA IV every 3-4 hours and 100 mg/kg EACA IV or PO every 6-8 hours; caution should be taken with

TXA in cats as it may induce seizures.[15, 16] Antifibrinolytics should be avoided in any hypercoagulable condition like DIC.

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