Disorders of Coagulation

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An Overview of Hemostasis

Clotting of blood occurs as a protection from exsanguination following injury. Injury occurs to blood vessel walls continuously (approximately 200,000 times/day), and so, therefore, does clotting of blood. Given that clotting is an ongoing process, there must be forces that serve to slow the activation of clotting factors in order to prevent thrombosis of the entire vascular system. The normal coagulation system represents a tightly controlled balance between procoagulant and anti-coagulant factors. The principal factors involved include the vascular endothelium, and the collagen underlying it, vascular tone, platelets, the clotting and fibrinolytic systems, and the flow characteristics of blood within the blood vessels.

A poorly functional coagulation system may predispose the patient to bleeding, and subsequent anemia, or pathological thrombosis of the vascular system. In humans, 60-70% of intensive care patients have laboratory evidence of a coagulopathy.

The ultimate goal of coagulation is the formation of the fibrin clot, the process of which is divided into local vasoconstriction, primary hemostasis (the formation of a platelet plug) and secondary hemostasis, which is responsible for the development of the fibrin clot.

Local Vasoconstriction

Following transection or injury to a small blood vessel, the blood vessel constricts, in some cases to the point that its lumen is obliterated. This intense vasoconstriction is mediated by release of seratonin, and other vasoconstrictors released from platelets, that adhere to the walls of damaged vessels.

Primary Hemostasis

- Primary hemostasis is provided by platelets. Exposure of sub-endothelial collagen and tissue factor results in rapid adhesion of platelets to the affected area. This process is mediated by von Willebrands factor (vWF). Locally, platelets release activating factors that result in clot growth. As platelet numbers increase, they aggregate to bridge the damaged zone in the blood vessel, and form a haemostatic plug. This plug is stabilized by a thrombin-mediated platelet fibrin meshwork, which traps platelets and red blood cells. Platelet contractile proteins further stabilize and consolidate the clot.
- Platelets aggregate and form the ***primary hemostatic plug***, which is very short-lived (seconds) and is unstable; but serves as a framework in which secondary hemostasis occurs.
- Further platelet activation allows exposure of specific surface receptors for VWF and fibrinogen. This localizes the reaction to the area of injury.
- Thromboxane A_2 release from activated platelets causes platelet aggregation and further blood vessel constriction at the site of injury.

- Soluble fibrin monomers released from an activated clotting cascade interact with vWF to allow further platelet incorporation into the platelet plug.

Secondary Hemostasis

- Secondary hemostasis involves activation of circulating coagulation factors to form fibrin. Coagulation factors circulate as inactive zymogens (enzymes).
- ***Intrinsic Clotting Cascade*** – factor XII is activated by contact with sub-epithelial collagen, and the platelet plug, which leads to the formation of fibrin via the following cascade.
- Factor XII activates factor XI, which activates IX; Factor IX combines with factor VIII to activate factor X. Factor X combines with factor V, calcium, and platelet phospholipid to convert factor II (prothrombin) to thrombin, which converts the soluble protein fibrinogen to an insoluble state (fibrin).
- ***Extrinsic Clotting Cascade*** – tissue trauma causes release of pro-coagulants (tissue thromboplastin) that activate factor VII, which activates factor X. Factor X combines with factor V, calcium and platelet phospholipid to convert factor II (prothrombin) to thrombin, which converts the soluble protein fibrinogen to an insoluble state (fibrin).
- ***Vitamin K_1 Dependant Factors*** are factor II, VII, IX and X.

Hemostatic Modulation

Hemostasis is a dynamic process – clotting of blood must be modulated or balanced by clot lysis and attenuation of the clotting cascades to avoid thrombosis of the entire vascular system. Modulation is a complex process involving dissolution of a blood clot, activation of naturally occurring anticoagulants, and inactivation of activated coagulation factors.

- The normal vascular endothelium is anti-thrombogenic.
- Contact of blood with tissue factor, sub-epithelial collagen, and the platelet plug also activates fibrinolytic (tissue plasminogen activator) and kinin pathways, leading to the production of plasminogen, which is converted to plasmin, which causes lysis of thrombi, and inhibition of clotting factor activation and platelet aggregation.
- Activation of antithrombin III, a protein synthesized in the liver also occurs. Antithrombin III is a cofactor for heparin, which inhibits activation of factors IX, X, and thrombin.
- Thrombin – At low concentrations, thrombin activates factor VIII: Ca complex, and factor V. At higher concentrations, thrombin inactivates activated factor V and factor VIII: Ca complex.
- The breakdown of fibrin leads to the generation of fibrin degradation products (FDP's) The fibrin generating potential of thrombin inhibited by FDP's and fibrin mediated thrombin absorption. FDP's interfere with normal platelet function and the action of thrombin.

Many other endogenous anti-thrombotic agents are responsible for down-regulating the clotting system to prevent excessive intravascular coagulation. A summary of naturally occurring endogenous anti-coagulants is presented in the following table.

Down-regulator of clotting	Pro-thrombotic target
Antithrombin	Factors Xa, XIIa, XIa, thrombin
Alpha-1 protease inhibitor	Factor XIa, elastase
Alpha-2 antiplasmin	Plasmin
Alpha-2 macroglobulin	Kallikrein, plasmin, thrombin
C1 inhibitor	Factor XIIa, kallikrein
Heparin cofactor II	Thrombin
Protein C	Factors VIIIa, Va
Tissue factor pathway inhibitor	Factor VIIa-tissue factor complex

Laboratory Evaluation of Hemostasis

There are numerous blood tests and clinical observations that may be used to evaluate hemostasis in a patient suspected of having a bleeding disorder. These are briefly outlined below. It is important to note that blood collection from patients suspected of having a bleeding disorder must be obtained with minimal restraint or excitement to the patient. Venipuncture should be clean, and preferably from a peripheral vein to minimize the chances of continuous bleeding following sample taking.

Activated Clotting Time (ACT) and Activated Partial Thromboplastin Time (APTT)

- The ACT and APTT evaluate the intrinsic and common clotting cascades.
- The ACT is measured using 2 ml of whole blood added to a tube containing diatomaceous earth, and incubated at 37.5°C until a clot is first seen. The normal range for the ACT in dogs ranges from 70-120 seconds, and in cats ranges from 60-90 seconds, although values up to 120 seconds may be considered normal in cats. It must be remembered that platelets are required to initiate the clotting cascade, and therefore patients with platelet deficiencies may also have a prolonged ACT. In addition, patients concurrently on treatment with synthetic colloids such as dextran 70 or pentaspan may also have a prolonged ACT by as much as 1.5 times normal. Patients with hypofibrinogenemia may have poor clot formation in an ACT tube, or multiple small clots.
- The APTT is more accurate in the detection of subtle abnormalities of the intrinsic and common clotting pathways as a clotting factor must be reduced below 30% of normal before APTT is prolonged.
- Diseases associated with prolongation of the ACT and APTT include liver disease, congenital coagulopathies, vitamin-K antagonist anticoagulant toxicity, and disseminated intravascular coagulopathy.

One-Stage Prothrombin Time (PT)

- The Prothrombin time evaluates the extrinsic and common clotting cascade.
- Citrated plasma is added to a thromboplastin-Calcium mixture.
- Prolongation of the prothrombin time is associated with liver disease, DIC and Vitamin K antagonism.

Thrombin Time (TT)

- Thrombin time assess the reactivity of fibrinogen to exogenous thrombin.
- Prolongation of thrombin time is associated with severe hypofibrinogenemia and dysfibrinogenemia.

Fibrinogen Degradation Products (FDP's)

- Fibrin degradation products are formed when plasmin degrades fibrin or fibrinogen.
- The presence of FDP's in circulating blood generally indicates the presence of disseminated intravascular coagulation (DIC). However, they may also be elevated in dogs and cats with venous or arterial thrombosis, intravascular hemolysis, and dogs with anticoagulant rodenticide toxicity.

Blood Smear Examination

- On a good quality blood smear there should be 10-25 platelets per high power field (HPF) using 40 X objective.
- Animals with a primary bleeding disorder caused by thrombocytopenia will have less than 2-5 platelets per HPF.

Buccal Mucosal Bleeding Time (BMBT)

- The BMBT evaluates the interaction between platelets and endothelium leading to the formation of the haemostatic plug, and is primarily used as a clotting test to evaluate platelet function, i.e., the BMBT is a test of primary hemostatic function.
- The test procedure involved measuring the time for blood to clot following a small incision in engorged mucus membranes.
- The normal time for BMBT is 1-3 minutes in dogs and cats.
- Prolongation of the BMBT will occur due to thrombocytopenia or platelet dysfunction (thrombocytopathia).

Hypocoagulation

Hypocoagulation may develop in animals for many reasons, and may involve failure of primary hemostasis, secondary hemostasis, or both.

Failure of Primary Hemostasis

Causes of Failure of Primary Hemostasis

Clinical signs of failure of primary hemostasis include petechiae, ecchymosis, and mucosal bleeding. Typically, platelet counts of less than 40×10^9 are required for spontaneous hemorrhage to occur, however counts of less than 100×10^9 may magnify other causes of hemorrhage.

Primary hemostatic disorders may result from the following:

- A decrease in platelet production.
- An increase in platelet destruction.
- An increase in platelet utilization.
- Altered platelet function (thrombocytopathia).

The causes of primary hemostatic defects are summarized as follows:

Thrombocytopenia	**Thrombocytopathia**
• Decreased production – Immune mediated megakaryocyte hypoplasia – Bone marrow aplasia – Drug induced megakaryocyte hypoplasia – (estrogen) – Myelophthisis – Cyclic thrombocytopenia • Incased destruction, utilization, sequestration – Immune mediated thrombocytopenia – Drug induced – Microangiopathy – Disseminated intravascular coagulopathy – Vasculitis – Splenomegaly, splenic torsion – Endotoxemia – Acute hepatic necrosis – Neoplasia – FeLV/FIV/ehrlichiosis	• Altered platelet function – hereditary – Von Willebrands disease – Canine thromboplastic thrombopathia – Canine thrombopathia – basset hounds, foxhound – Collagen deficiency diseases • Altered platelet function – acquired – Drugs – antiprostaglandin therapy, antibiotics, phenothiazines, modified live vaccines – Disease states that alter clotting of blood * Systemic lupus erythematosus * Renal disease * Liver disease – Myeloproliferative disease – Dysproteinemia

Incidence

In dogs, approximately 5% of dogs with thrombocytopenia have immune-mediated thrombocytopenia, 13% have thrombocytopenia caused by neoplasia, 23% have thrombocytopenia caused by infectious or inflammatory causes, and over 50% of dogs have thrombocytopenia caused by unidentified causes or miscellaneous diseases, such as pulmonary or cardiac disease.

In cats with thrombocytopenia, 2% have immune-mediated disease, 20% have neoplasia, 30% have infectious causes, 7% have cardiac disease, 22% have multiple causes, and the remaining 20% of causes are undetermined.

The Approach to the Patient with a Disorder of Primary Hemostasis

History

- Hemorrhage is an inconsistent sign on presentation.
- Question access of the patient to toxins (see below), drugs, human medications, and any previous illness, including adverse drug reactions and response to treatment.

- Immune-mediated disease is more prevalent in middle aged, pure bred, female dogs, but may be seen in younger and older patients.
- Immune-mediated thrombocytopenia has an increase in prevalence in Cocker Spaniels, German Shepherds, Poodles, and Old English Sheepdogs.
- The presence of concurrent illness is important – review all body systems during history taking to aid in determining the presence or absence of cardiac, respiratory, gastrointestinal, renal or endocrine disorders.

Clinical Presentation

- Hemorrhage as a clinical sign is inconsistently associated with thrombocytopenia and thrombocytopathia. Most patients are presented for lethargy, weakness, pyrexia, or symptoms associated with the underlying cause of the primary clotting disorder.
- Clinical bleeding generally occurs when platelet count is below 30-50 x 10^9/l.
- The animal is unable to form a primary hemostatic plug, however, the secondary plug will eventually cover the damaged blood vessel with fibrin to stop the bleeding. Clinically, this is seen as multiple, short-lived bleeds that are arrested as soon as fibrin is formed, i.e., multiple superficial hemorrhages.
- Petechiae, ecchymosis, bleeding from mucosal surfaces.
- Malena, epistaxis, hematochezia, and occasionally hematuria are seen clinically.
- Splenomegaly, and/or hepatomegaly may be associated with platelet sequestration (hypotension, shock, hypothermia, endotoxemia) phagocytosis (immune-mediated disorders), or neoplasia.

Laboratory Abnormalities in Primary Hemostatic Disorders

- Blood smear – thrombocytopenia may be evident. Look at a blood smear under the 40 and 100X objective lenses under oil immersion. Evaluate the smear for the presence of large platelets, and megakaryocytes that may suggest a bone marrow response, and signs of a regenerative or non-regenerative anemia. Remember, it may take 3-5 days for signs of a regenerative anemia to appear following the onset of clinically significant hemorrhage. Useful hints on blood smear evaluation include:
  - 6-7 platelets per HPF = 100x10^9/l
  - < 3-4 per oil field = significant thrombocytopenia
  - < 1 platelet per 50 erythrocytes suggests thrombocytopenia
- BMBT – prolonged.
- ACT – may be normal. Mild increases in ACT may be seen in cases of von Willebrands disease, or severe thrombocytopenia or thrombocytopathia.
- PT/APTT – normal; APTT may be prolonged in von Willebrands disease, thrombocytopenia or thrombocytopathia.
- CBC:
  - May show evidence of a regenerative anemia if the disease process has been present for greater than 3-5 days.
  - Spherocytes may be seen if a concurrent immune-mediated hemolytic anemia is present.

- The presence of leukopenia and/or a non-regenerative anemia may be seen with FIV/FeLV and FIP.
- The presence of neutrophilia may suggest inflammation, or a reactive response from the bone marrow due to erythropoietin release secondary to anemia.
- The presence of neutropenia may suggest bone marrow disease, or increased tissue utilization.
- Chronic inflammatory disease may result in a non-regenerative anemia secondary to iron sequestration.
- Red blood cell morphology may give clues to the mechanism of thrombocytopenia in some disease states, e.g., the presence of shistocytes may suggest DIC or microangiopathic disease.

Thrombocytopenia

Approach to diagnosis

- Collect and save an EDTA and serum sample prior to treatment. These samples are required to establish baseline parameters for subsequent monitoring, and also provide the best diagnostic specimens for laboratory evaluation.
- Blood smear – evaluate platelet count (normal 10-25/HPF), and evaluate red cells for the presence of parasites (Hemobartonellosis, Babesia).
- PCV/TP – the presence of anemia may suggest Evans Syndrome, or may be secondary to bleeding. Note the color of the serum also – jaundiced serum or red/brown serum can be suggestive of hemolysis. This may aid in determining if the patient has a concurrent immune-mediated hemolytic anemia, DIC or other cause of hemolysis.
- FeLV/FIV should be tested in cats.
- Bone marrow biopsy – bone marrow biopsy is usually indicated in patients that have a pancytopenia, or in those patients not responding to conventional medical therapy.
- Coagulation profile – ACT, APTT, PT and FDP's should be performed to determine if the patient has an accompanying secondary clotting disorder.
- Drug history – assume thrombocytopenia to be the result of drug therapy until proven otherwise; discontinue all non-essential medications.
- Radiographic/ultrasonic evaluation of the abdomen and thoracic cavities is required to determine the presence of neoplasia, particularly in the heart, lungs, liver, spleen, kidneys and adrenal glands.
- Serum anti-nuclear antibody titer may rule out the presence of SLE.
- Detection of anti-platelet antibodies (platelet factor 3 test; megakaryocyte direct immunofluorescence assay) have limited availability, sensitivity, and specificity. Flow cytometry assays for detecting platelet-bound IgG has a high sensitivity – a negative test excludes a diagnosis of ITP/IMT; a positive test may indicated ITP/IMT, tumor-associated antigens on platelets, or drug-induced immune complexes on platelets.

Treatment

- **GOALS:** Stop bleeding, halt platelet destruction, correct underlying disorder.
- Administration of fresh whole blood, and platelet rich transfusions is typically unrewarding.
- Anticoagulant therapy in DIC (see later).
- Gut protectants such as H_2 antagonists and anti-emetics may reduce nausea in some patients.
- Glucocorticoids are used in immune-mediated disease.
- Pathogen specific drug therapy; removal of neoplasia, chemotherapy for neoplasia, treatment of cardiac disease, etc, depending on the underlying cause of platelet dysfunction.

Thrombocytopenia – Immune-mediated Thrombocytopenia

Characteristics

- Middle aged, female, purebred (English Sheepdog, poodles, German Shepherds, Cocker Spaniels) dogs are predisposed, but any breed or sex may be affected.
- Thrombocytopenia.
- Anemia may be present due to blood loss; regenerative if disease process has been present for longer than 3-5 days.
- Leukocytosis with left shift – due to erythropoietin release secondary to anemia, sepsis, or tissue inflammation secondary to infection, neoplasia, or hypoxia.
- Red cell morphology – spherocytosis (IMHA), shistocytes (DIC, vasculitis).
- Buccal Mucosal Bleeding Time (BMBT) – prolonged if platelet count below 50 x 10^9/l.
- NOTE – immune mediated thrombocytopenia is a diagnosis by exclusion. Drug induced and infectious, septic, and neoplastic causes of thrombocytopenia need to be ruled out first.

Treatment

- Immunosuppressive doses of prednisolone – 2-4 mg/kg PO q 12 hrs are the initial treatment of choice. High dose intravenous methylprednisolone sodium succinate (10-30 mg/kg IV) may be used if the patient is not able to tolerate oral medications on presentation. Glucocorticoids act primarily by inhibiting macrophage destruction of antibody-sensitized platelets. The also increase capillary resistance to hemorrhage, increase endothelial cell size and protein synthesis, and reduce production of prostacycline by vascular endothelial cells.
- Transfuse with whole blood if indicated by the patients condition and/or severity of anemia present. The activated clotting time is frequently prolonged due to thrombocytopenia, and usually does not indicate transfusion with fresh frozen plasma is indicated. However, recent evidence suggests that patients with severe thrombocytopenia benefit from provision of plasma, as they significantly reduce clotting times, and the severity of blood loss in patients with thrombocytopenia. We therefore recommend administration of fresh frozen plasma in severely thrombocytopenic patients with clinical signs of ongoing bleeding, falling PCV and associated morbidity.

- Additional therapy that has been advocated in the management of immune-mediated thrombocytopenia includes Azothiaprine 2 mg/kg PO q 24 hrs, cyclophosphamide 1.5-2.5 mg/kg PO q 24 hrs 4 days per week, or vincristine 0.02 mg/kg IV q 7 days.
- Currently, vincristine is the favored adjunctive agent. Vincristine 0.5-mg/sq. m IV causes megakaryocyte endomitosis and early release of platelets from the bone marrow. Vinca alkaloids such as vincristine bind to tubulin within platelets. This binding may make platelets less active following treatment with vincristine. However, when vincristine-filled platelets are phagocytosed by macrophages, vincristine is released into the cytoplasm of the macrophages, which results in macrophage death. The net effect of this is to reduce platelet destruction. Administration of combined vincristine and prednisolone is associated with a more rapid increase in platelet numbers, and shortened duration of hospitalization in dogs with IMT, when compared with the use of prednisolone alone. Early use of vincristine seems warranted in dogs with severe primary immune-mediated thrombocytopenia.
- Cyclophosphamide may be used in place of vincristine. In humans, cyclophosphamide has been used successfully in patients refractory to high dose corticosteroid therapy, and as part of a "rescue" protocol for patients that have a relapse or recurrence of thrombocytopenia. In a retrospective study on the use of corticosteroids in combination with either vincristine or cyclophosphamide in dogs, patients given vincristine had a more favorable outcome.
- Danazol, a synthetic androgen is reported to have synergism with prednisolone in the management of immune-mediated thrombocytopenia. Androgens reduce the number of Fc receptors on macrophages, reducing the rate of phagocytosis. Danazol also displaces glucocorticoids from globulins, thereby increasing serum glucocorticoid levels in the blood. The dose is 2-5 mg/kg PO q 12 hrs.
- Azothiaprine – impairs lymphocyte mitogenesis and immunoglobulin production. Response rates are similar to those obtained with cyclophosphamide. Efficacy in dogs is not well documented. The dose is 2 mg/kg PO q 24 hrs. Response times are generally 2-10 weeks following starting of treatment.
- Cyclosporine A is emerging as a valuable agent in the management of refractory cases of immune-mediated thrombocytopenia, and should be considered if patients are not responding to conventional therapy within 2-3 weeks. Cyclosporine A inhibits function of T-lymphocytes by inhibiting calcium-dependant transcription of interleukin-2. Cyclosporine A is also recommended in patients that have relapsed following treatment with prednisolone. The dose is 15 mg/kg q 24 hrs.
- Human Immunoglobulin – human immunoglobulin blocks Fc-receptors on macrophages, and is widely used as an emergency therapy in humans with immune-mediated thrombocytopenia. This treatment has been used in isolated cases in dogs, and is currently undergoing trials in dogs.
- Splenectomy is commonly used in humans with immune-mediated thrombocytopenia. Splenectomy produces remissions of up to 50-70% of human patients evaluated up to 5 years following surgery, as opposed to remission rates of 5-30% with medical therapy alone. The incidence of side effects of therapy with splenectomy is very low (5%) when compared to medical therapy (35%). In addition, following splenectomy, mean platelet life is nearly normalized in human patients with IMT, whereas mean platelet life did not return to normal in patients treated with prednisolone alone. There are very few reports on the benefit of splenectomy in animals with IMT. However, splenectomy should be considered a therapeutic option in patients with splenomegaly, and refractory immune-mediated thrombocytopenia.

- Platelet transfusion – 1 unit of platelet-rich plasma per 10 kg will raise platelet count by 40 x 10^9/l, or whole blood at 10 ml/kg will raise platelet count by 10 x 10^9/l. Administer every 1-2 days.

Thrombocytopathia

Etiology

The causes of thrombocytopathia/altered platelet function are listed below:

Inherited Thrombocytopathia	Disease-associated Thrombocytopathia	Drug-associated Thrombocytopathia
1. Von Willebrands Disease (a) Type I (b) Type II (c) Type III	1. Anemia – alters blood viscosity 2. Sepsis and DIC – alters platelet reactivity 3. Liver disease 4. Dysproteinemia – lymphocytic leukemia, multiple myeloma, macroglobulinemia, polyclonal gammopathies – increase blood viscosity and alter platelet adherence to blood vessel walls 5. Uremia – alters prostaglandin metabolism and reactivity of platelets	1. Antibiotics (a) Carbenicillin (b) Cephalothin (c) Moxolactam (d) Sulphonamides 2. Anti-prostaglandins[1] (a) Aspirin (b) Ibuprofen (c) Phenylbutazone (d) Naproxen 3. Cardiac/Respiratory (a) Aminophylline[2] (b) Isoproterenol (c) Propanolol (d) Theophylline[2] (e) Verapamil[3] 4. Miscellaneous (a) Barbituates[4] (b) Dextran 70, pentaspan (c) Heparin

[1] Aspirin causes irreversible acetylation of platelet cyclo-oxygenase enzyme. The remaining antiprostaglandin drugs result in reversible inhibition of prostaglandin metabolites.

[2] Inhibition of phosphodiesterase causes increased intra-platelet cAMP.

[3] Signal transduction affected due to interference with the rise of intra-platelet calcium levels.

[4] Membrane MOA is by interference or interaction with platelet membrane receptors.

Diagnosis of Platelet Dysfunction

- Evaluate platelet numbers by automated counts and blood smear evaluation.
- Determine plasma von Willebrands Factor concentration.
- Determine drug history to rule out exposure to drug therapy that may result in thrombocytopathia.
- Blood test – CBC, biochemistry profile aids in determining disease processes likely to impair platelet function.
- Perform BMBT.
- Perform ACT, APTT, PT.
- Perform FDP's, AT III levels.

Thrombocytopathia – Von Willebrand's Disease

- Von Willebrand's disease is the most common inherited bleeding disorder in dogs.
- Caused by a lack of von Willebrand's factor (vWF). VWF is an adhesive glycoprotein produced by endothelial cells and megakaryocytes. Von Willebrand's factor is present in different glycoprotein sizes, called multimeres. The larger multimeres are more effective in promoting platelet adhesiveness than smaller multimeres.
- Von Willebrand's factor promotes the adhesion of platelets to exposed vascular sub-endothelium, and increased platelet-to-platelet adhesiveness by adhering to platelet von-Willebrand's receptors. Von Willebrand's factor also forms a tightly bound complex with factor VIII, thereby prolonging the half-life of factor VIII.

Type I von Willebrand's Disease

- Most common form of von Willebrand's disease.
- All multimeres of von Willebrand's factor are present, but are dramatically reduced in number.
- Identified in more than 50 breeds of dog.
- Doberman, Standard Poodle, Shetland Sheepdog, German Shepherd.
- Clinical signs are most commonly associated with prolonged surgical bleeding.
- Stress and vaccination may cause transient thrombocytopenia, and result in clinical bleeding.

Type II von Willebrand's Disease

- Larger multimeres are absent in these patients, which can result in severe bleeding.
- German Wirehaired Pointer, German Shorthaired Pointer.

Type III von Willebrand's Disease

- All multimeres are absent.
- Life-threatening hemorrhagic episodes.

- Patients with type III von Willebrand's disease not uncommonly die at birth, or are seen as puppies or kittens with fading puppy or kitten syndrome and die during the first few days of birth.
- Chesapeake Bay Retrievers, Scottish Terriers, Shetland Sheepdog.

NOTE: strenuous exercise, epinephrine, pregnancy, and stress raise levels of vWF; recent studies have shown no association between hypothyroidism and acquired von Willebrand's disease.

Clinical Signs

- Petechiae, ecchymosis, mucosal bleeding are rare signs.
- Intra-operative bleeding.
- Occasional bleeding into body cavities, hematoma formation.
- Increased perinatal mortality.

Laboratory Evaluation and Diagnosis

- Cuticle bleeding time, and buccal mucosa bleeding time are prolonged in patients with von Willebrand's Disease.
- ACT/APTT/PT are usually normal. Occasionally APTT will be prolonged if the patient has a concurrent partial factor VIII deficiency.

Therapy of von Willebrand's Disease

- Administration of cryoprecipitate increases vWF levels significantly within 30 minutes of administration to dogs with type I vWD. The effect lasts 4 hours.
- DDAVP (desmopressin acetate) (trade name "Minirin") causes release of stored vWF from endothelial cells. Dose is 1-4 ug/kg SC, onset of activity is 30 minutes post administration; duration of activity is 2 hours. This is the most useful therapy in a practice situation prior to anesthesia, or where blood donors or blood products are not readily available.

Thrombocytopathia – Salicylate Toxicity

Salicylates – Pharmacology

- Salicylates are salts or esters of salicylic acid.
- Salicylates are metabolized in the liver by glucoronyl transferase, and conjugated to glucoronic acid.
- Neonates and cats have low levels of this enzyme.
- Salicylates are excreted by the kidney, via glomerular filtration, and proximal tubular secretion.
- Salicylates irreversibly inhibit cyclo-oxygenase enzyme. Cyclo-oxygenase catalyses synthesis of endoperoxides and thromboxane in platelets, which induce platelet release and aggregation. Because platelets do not have nuclei, they are unable to synthesize new cyclo-oxygenase enzymes, and therefore, following administration of salicylates, have

reduced production of thromboxane and endoperoxides. This in turn, leads to a decrease in platelet activity.

- Endothelial cells do have nuclei, and can synthesize new cyclo-oxygenase enzymes. Endothelial cells produce PGI_2, which is a potent inhibitor of platelet aggregation.
- The combination of these two effects results in clinical bleeding, especially in the presence of predisposing factors such as von Willebrand's Disease.

Thrombocytopathia – Antibiotic Chemotherapy Agents

Penicillin, Carbenicillin, Ampicillin

- Inhibit platelet aggregation by impairing collagen and vWF (risocetin) -induced platelet aggregation.

Moxolactam

- Moxolactam is a beta-lactam antibiotic. Moxolactam induces a coagulopathy by binding to platelets, causing interference with the development of ADP-lectan binding, which is required for platelet aggregation. This most commonly occurs following 2-3 days of therapy.
- Moxolactam also decreases growth of gastrointestinal flora, depressing absorption of vitamin K.

Thrombocytopathia – Hypercoagulation-Induced Thrombocytopathia and DIC

These conditions will be reviewed under the heading "Combined primary and secondary coagulopathies".

Secondary Hemostatic Disorders

Failure of secondary hemostasis may result from inherited or acquired factor deficiencies. Isolated inherited factor deficiencies are rare, but have been reported. Most commonly these occur in young animals, with minimal or no history of trauma. Blood clotting factor deficiencies are usually acquired. Some of the most common causes are:

- Anticoagulant rodenticide toxicity (prevents production of functional factors II, VII, IX, X).
- Disseminated intravascular coagulopathy.
- Liver failure – results in decreased clotting factor production, decreased clearance of fibrin degradation products (FDP's), and a relative vitamin K deficiency (also seen with cholestasis).
- Hypothermia – slows activity of enzymes involved in the coagulation cascade.
- Factor loss – through excessive consumption with thrombocytopenia, or thrombocytopathia.
- Dilution of clotting factors– following fluid therapy, diluting total solids to less than 35 g/L.

Vitamin K Deficiency and Antagonism

Pathophysiology

- Vitamin K is required for the post-ribosomal carboxylation of glutamyl residues of factors II, VII, IX and X.
- Vitamin K also required for the synthesis of Protein C (Inhibitor of Factor V and Factor VIII).
- Anticoagulant rodenticides inhibit the enzyme vitamin K epoxide reductase, thereby preventing recycling of Vitamin K epoxide to the biologically active vitamin K_1. Under normal conditions, vitamin K_1 carboxylates clotting factors II, VII, IX and X, which allows them to bind calcium, and participate in the clotting system. Vitamin K_1 deficiency therefore reduces the carboxylation of the dependent clotting factors, and results in a secondary coagulopathy.
- With Vitamin K_1 deficiency or antagonism, the liver produces inactive coagulation proteins, which are antigenically similar to factors II, VII, IX and X.
- The half life of vitamin k-dependant clotting factors is as follows:
  - Factor II – 41 hrs
  - Factor VII – 7 hrs
  - Factor IX – 14 hrs
  - Factor X – 16.5 hrs
- Peak serum concentration occurs 12 hours after experimental exposure to vitamin K antagonists.

Clinical Signs

- Clinical signs are generally related to the presence of bleeding into organs, resulting in organ dysfunction, and the presence of bleeding into body cavities.
- Acute death without previous signs of illness can occur with a sudden haemorrhage into the brain, pericardial sac or thoracic cavity.
- Dyspnea may occur due to anemia, hemorrhage into the lung parenchyma, anemia, intrathoracic bleeding and intra-abdominal hemorrhage.
- Anemia, weakness, pallor, hematemesis, epistaxis, and malena may occur.
- Hematomas may form at sites of trauma and venipuncture.
- Lameness may occur secondary to hemorrhage into joints and muscle tissue.
- ACT, APTT and PT are prolonged.
- The most sensitive early indicator of exposure to a toxic dose of a vitamin k antagonist is the prothrombin time. The reason for this is that the prothrombin time tests activity of factor VII, which has the shortest half-life of all of the vitamin k-dependant factors. Prothrombin time will become prolonged 36 hrs following exposure to vitamin K antagonists. APTT and ACT will become prolonged approximately three days post exposure.

Treatment

- Provide oxygen supplementation if indicated.
- Provide ventilatory assistance if the patient is not ventilating adequately. Perform bilateral thoracocentesis to drain pleural fluid if indicated by the presence of expiratory dyspnea, biphasic expiration, and reduced lung sounds.
- Provide circulatory support – patients showing signs of decompensating response to anemia (elevated heart rate at rest, tachycardia, depression, lethargy, recumbency and hypoxia), or patients with a PCV less than 15% should receive transfusion of whole blood.
- Patients with a significant increase in ACT, PT or APTT should receive transfusion of fresh frozen plasma.
- Begin therapy with vitamin K_1 at 2-5 mg/kg/day. If the patient will tolerate oral medication with food, begin therapy with oral vitamin K_1. If the patient will not tolerate oral medication with food, begin with injectable vitamin K_1. Remember, these patients have a coagulopathy – the fewer injections they get the better! Vitamin K_1 has greatest bioavailability if given orally and fed with a fatty meal. Treatment should continue for 10-35 days, depending on whether a first, second, or third generation vitamin K antagonist has been ingested. A clotting profile (APTT, PT) should be carried out 3 days following cessation of vitamin K_1 therapy to determine if a more prolonged course of treatment is required. It must be noted that vitamin K_1 will take 12-36 hrs to normalize clotting times in dogs and cats. These patients will continue to bleed during this lag phase of treatment – they must be kept quiet and preferably under observation, and provided with cardiovascular and respiratory support if dictated by the patients condition.

Liver Disease

Pathophysiology

- The liver is the site of synthesis of most coagulation factors and their inhibitors.
- Patients with liver disease may develop coagulopathies in situations of acute fulminating hepatopathy or acute hepatic necrosis, or with loss of greater than 70% of hepatocellular mass. In both of these situations, a reduction in functional hepatocellular mass reduces production of clotting factors, and reduces clearance of activated clotting factors and fibrin degradation products and availability of vitamin K.
- DIC may occur due to systemic dissemination of the bi-products of hepatic inflammation.
- Increased plasmin generation in liver disease promotes kinin activation, which causes hypotension, shock and end organ damage.
- Reduced synthesis of ATIII and reduced clearance of activated clotting factors provides a stimulus for thrombus formation. Renal or pulmonary thromboses are potential complications.

Hypercoagulation

Definition

Hypercoagulation is an imbalance in hemostasis, with an increased propensity for thrombus formation. Thrombosis depends of three major risk factors:

- Changes in the vessel wall (vascular injury).
- Impairment of blood flow (blood flow stasis).
- Alterations in blood constituents (hypercoagulability).

This concept is known as **Virchow's triad**, and is fundamental to the understanding of thromboembolism and its prevention.

- **Vascular injury** – leads to exposure of sub-endothelial collagen, resulting in platelet adhesion, and activation of the contact phase of coagulation. Vascular injury may be caused by the following:
  - Trauma.
  - Catheterization.
  - Inflammatory disease.
  - Neoplastic invasion.
  - Parasitic damage.
  - Plaque deposition (amyloidosis, arteriosclerosis).
- **Vascular stasis** favors thrombosis by retarding local clearance of activated clotting factors, and by causing local tissue hypoxia and vascular injury (through ATP depletion and cellular dysfunction). Vascular stasis may result from the following:
  - Hypovolemia.
  - Shock.
  - Cardiac insufficiency.
  - Blood vessel compression (e.g., GDV, neoplasia, organomegaly, etc.).
  - Immobility.
  - Hyperviscosity (dehydration, polycythemia, leukemia, hyperglobulinemia, hyperfibrinogenemia).
- **Hypercoagulability** – results from an imbalance between the procoagulants (platelets, coagulation proteins), and the anticoagulants (natural anticoagulants, fibrinolytic system). Hypercoagulability may therefore result from platelet hyper-aggregability, excessive activation or decreased removal of coagulation factors, deficiencies of natural anticoagulants, or defective fibrinolysis.
  - Platelet aggregation – platelet adhesion to damaged vascular endothelium is facilitated by von Willebrand's Factor (vWF), and glycoproteins (platelet activating factor etc). Following adhesion, platelets produce and release pro-aggregating substances, including thromboxanes A_2, ADP, and prostaglandins G_2, and H_2. These induce shape changes in platelets, and the expression of glycoproteins IIb and IIIa receptors on the platelet surface, which allow binding of fibrinogen to platelets. The endothelium releases inhibitors of platelet aggregation including prostacycline (PGI-2), ADPase, nitric oxide (NO) to balance the clotting system.

Increased activation of coagulation factors (by vascular injury or inflammatory mediators) and decreased removal of factors from an area of injury (due to stasis or decreased activity of the reticuloendothelial system) may contribute to thrombosis.

Hypercoagulable States and DIC

Primary hypercoagulable states are inherited disorders that have not been reported in dogs or cats to date.

Secondary hypercoagulable states include the following:

- **Nephrotic Syndrome** – Nephrotic syndrome typically leads to a loss of small molecular weight proteins such as albumin, antithrombin III, and macroglobulins into the urine, and retention of larger molecular weight proteins such as procoagulant proteins, and macroglobulins. Glomerular loss of antithrombin III, combined with retention of procoagulant proteins such as factor VIII, fibrinogen, and fibrinectin, leads to a hyper-coagulable state. In addition, loss of albumin results in platelet hyperaggregability. Hypercholesterolemia present in nephrotic syndrome increases blood viscosity and platelet hypersensitivity. The net result is a hyper-coagulable state within the intravascular space. Decreased antithrombin III levels also results in increased hepatic production of alpha-2 macroglobulins, which inhibit hemostasis.
- **Neoplasia** – vascular stasis due to tumor compression and vascular injury, platelet activation by tumor cells, release of tissue thromboplastin by tumor cells and by mononuclear cells stimulated by tumor antigen, elevated fibrinogen, and hypo-fibrinolysis all may induce a hypercoagulable state.
- **Acute pancreatic necrosis** – may cause hypercoagulation through several mechanisms, including increased serum levels of acute phase proteins (esp. fibrinogen), the presence of pancreatic vasculitis, vascular stasis, hypercholesterolemia, and hypo-fibrinolysis.
- **Immune-mediated hemolytic anemia** – Incidence of pulmonary thromboembolism in dogs with IMHA is 11-33%. Mechanisms inducing hypercoagulability include endothelial-mediated and monocyte-amplified reactions triggered by anti-erythrocyte antibodies, acute phase reactants, vasculitis, indwelling intravenous catheters, and corticosteroid therapy.
- **Hypercortisolemia** – the pathogenesis of hypercoagulation in Hypercortisolemia includes hypofibrinolysis secondary to increased activity of plasminogen activator inhibitor and alpha-2 antiplasmin; and increased levels of coagulation factors (esp. factor VIII).
- **Arteriosclerosis** – rare in dogs; may be found in hypothyroidism.
- **Diabetes mellitus** – hypercoagulability occurs secondary to increased platelet aggregability due to decreased release of prostacycline, reduced platelet sensitivity to prostacycline, and increased production of thromboxanes.
- **Sepsis and Multiple Organ Dysfunction Syndrome** – activation of coagulation is a normal component of the acute inflammatory response. Inflammatory cytokines alter the endothelium, cause release of tissue factor, and stimulate production of platelet activating factor. The fibrinolytic pathway is also initially activated, but is subsequently inhibited, primarily due to increases the plasminogen activator inhibitor – an acute phase protein. When these processes involve systemic or widespread tissue injury, a hypercoagulable state results.

Diagnosis of Hypercoagulation

Diagnosis of hypercoagulation is made based on the following:

- The clinical setting – see causes of hypercoagulable states as listed above.
- Routine screening tests – note that shortened coagulation times does not imply a thrombotic state, as normal individuals may also have shortened coagulation times.
- Fibrin degradation products – are generated by the dissolution of fibrin by plasmin. Increased levels may be seen with increased thrombus formation, alterations in fibrinolytic activity, and alterations in hepatic clearance.
- Antithrombin III levels – may indicate thrombotic tendency when levels are decreased. However, few thrombotic states result from primary antithrombin deficiency.
- D-dimer – Is a fibrin degradation product formed when cross-linked fibrin is proteolyzed by plasmin. Since cross-linkage of fibrin implies the production of fibrin, elevations in D-dimer imply the presence of fibrin and circulating plasmin. Sensitivity of the test is 75-93%; specificity of the test is 70-77%.

Clinical Manifestations of Hypercoagulation

The clinical manifestation of imbalance between pro-thrombotic and anti-thrombotic forces that occurs in patients with predisposing causes of hypercoagulation outlined above is pathologic thrombosis. Pathologic thrombosis can manifest on a macroscopic or microscopic basis.

The clinical manifestation of hypercoagulation may be overt bleeding as in disseminated intravascular coagulation, or no bleeding, as with pulmonary thromboembolism. Therefore, even though the manifestations of hypercoagulation are the opposite, the underlying cause is the same – excessive activation of the clotting cascade.

In hypercoagulable states, the production of thrombin may be as much as 100 times greater than the non-injury rate. Production of thrombin is greatest following severe trauma. In addition to the increased circulating thrombin, concentrations of antithrombin III and protein C are significantly reduced. The result is a strongly pro-thrombotic environment in the systemic circulation, resulting in disseminated intravascular coagulation. Once this occurs, the fibrinolytic system is activated to prevent the microcirculation from becoming clogged with microthrombi. As the fibrinolytic system lyses the pathologic thrombi, it also lyses non-pathologic thrombi at sites of vascular injury, causing further activation of the clotting cascade due to exposure of sub-endothelial collagen and tissue thromboplastin. This causes further clot deposition, and finally, depletion of the pro-coagulant factors, and the bleeding associated with disseminated intravascular coagulation.

Not all patients with significant tissue injury develop disseminated intravascular coagulation (DIC). The likelihood of the development of DIC is determined by the volume of tissue thromboplastin released into the circulatory system, and the patients' vascular volume status (i.e., blood flow through capillary beds, and hence oxygen delivery to tissues).

Hypercoagulable States – The Clinical Approach

Primary Inducers of Hypercoagulation

Intravascular Hemolysis	Viremia	Neoplasia
• Hemolytic transfusion reaction • Hemolytic anemia **Septicemia** • Gram-Negative bacteria (endotoxin) • *E.coli* • *P.Hemolytica & P.multocida* • *Salmonella spp.* • Gram-positive bacteria (bacterial coat mucopolysaccharide) • *Staph spp.* • *Strep spp.* • *Clostridium spp.* • *Mycobacterium spp.*	• Infectious canine hepatitis • CDV • Canine herpes virus • FIP • Feline panleukopenia **Parasitic Infections** • Protozoal infection • Metazoan infection **Obstetric Complications** **Miscellaneous** • Gastric dilatation-volvulus • Diabetes mellitus	**Massive Tissue Injury** • Burns • Trauma • Surgical procedures • Heat stroke **Venoms and Toxins** • Snake bite • Insect stings • Aflatoxin **Hepatic Disease** **Pancreatitis**

From the above table, a good rule of thumb is "Expect DIC in patients who have significant hypotensive crisis, impaired blood flow to a major organ, or release of vasoactive substances into the vasculature". It might seem that this approach may lead to a daily expectation of DIC. However, failure to expect hypercoagulation and DIC will result in increasing patient morbidity and mortality. Hypercoagulation and DIC are much easier to prevent than they are to treat!

Clinical Signs of Hypercoagulation

The clinical signs of hypercoagulation are usually referable to the underlying disease, the presence of pathological vascular thrombosis, or bleeding. Symptoms may have the following characteristics:

- May be peracute, acute, or chronic depending on whether the underlying illness is acute or chronic.
- Laboratory changes, with no clinical signs may be present with a peracute hypercoagulopathy.
- Acute DIC will be evidenced by oozing from venipuncture sites, mucous membrane hemorrhage, petechiae, ecchymosis, purpura, hematoma formation, and hemarthrosis.
- Physical findings in acute and peracute disease are associated with decreased organ perfusion.
- Organ Dysfunction and MODS is a consequence of acute DIC. Any organ can be affected by a coagulation disorder. Hepatic necrosis is common, renal thrombosis or micro-thrombosis may result in renal dysfunction (renal failure). Gastric ulceration and submucosal necrosis result from gastrointestinal thrombi, the clinical signs of which include hematemesis, malena,

hematochezia, and occult fecal blood. Impaired pulmonary function may occur due to microvascular thrombosis, the clinical signs of which include tachypnea, hypoxia, and the development of acute respiratory distress syndrome (ARDS). Cerebral microvascular thrombosis may result in altered mentation or consciousness, convulsion or coma.

- Chronic DIC develops with an illness producing low grade or intermittent procoagulant release stimulus. This enables clotting factors, anticoagulant proteins, and platelets to be replenished. Chronic DIC is most commonly associated with neoplasia of the vascular system or soft tissues, e.g., hemangiosarcoma, pheochromocytoma, mast cell neoplasia, lymphoma etc.

Diagnosis of Hypercoagulopathy and DIC

- Diagnosis is based on clinical suspicion, knowledge of associated diseases and serial laboratory coagulation tests. Early recognition is essential as treatment is more effective when administered early in the disease process. Note – hypercoagulation and DIC occurs in animals with dynamic disease processes – CHANGES OCCUR RAPIDLY. Repeated patient evaluation is recommended on a regular basis – i.e., every 3-4 hours.

Shistocytes

- Result from mechanical damage to red cell membrane from microvascular fibrin strands. Shistocytes are more commonly found in patients with chronic or compensated DIC.

Thrombocytopenia-Thrombocytopathia

- Platelet counts are variable in DIC. Repeat blood smears essential. Platelet counts in the low-normal interval in patients with severe systemic inflammation are suspected to have DIC, or are expected to develop DIC, particularly if mega-platelets are seen.
- Buccal mucosal bleeding time is usually ineffective in determining thrombocytopathia, due to the variability of platelet counts in patients with hypercoagulation and underlying diseases.

Fibrinogen

- DIC results in consumption of fibrinogen when the coagulation cascade is activated and fibrinogen is biotransformed to fibrin.
- Fibrinogen is an acute phase inflammatory protein and will be increased with acute focal or systemic inflammation.
- A low-normal concentration of fibrin in patients with systemic inflammation is supportive of DIC.
- A decrease in fibrinogen often precedes a thrombocytopenia.
- In the dog, fibrinogen levels below 75 mg/dl will result in prolongation of the APTT and PT.

Prothrombin Time, APTT and ACT

- The Activated Clotting Time is the most useful test for detecting fulminant DIC in clinical practice. The APTT and PT can be used to detect low grade, chronic, or compensated DIC, as they are more sensitive tests than the activated clotting time.

Fibrin Degradation Products (FDP's)

- Result from plasmin degradation of fibrin and fibrinogen. FDP's are composed of fibrin fragments X, Y, D, and E. The commercial tests detect E and D.
- Physiologically increased FDP's associated with increased bleeding tendency as they act as anticoagulants preventing biotransformation of fibrinogen to fibrin.
- The presence of FDP's not pathognomic for DIC, as they are also resent in hepatic failure, major focal vascular thrombosis, dysfibrinogenemia, and excessive fibrinolysis.

D-Dimer tests detect the D-dimer of fibrin degradation. The presence of D-Dimer in blood implies fibrinolytic activity secondary to coagulation. **D**-dimer tests are commercially available as a snap test.

Antithrombin III (AT III)

- ATIII is a α-2-macroglobulin acute phase protein manufactured in the liver, that inhibits serine (amino acid) proteases in the coagulation pathways (Factors XII, XI, X, IX, II).
- Patients in a hypercoagulable state, that are actively converting prothrombin to thrombin, will have a low AT III concentration. Affinity of AT III for the serine proteases is increased up to 100-fold by heparin. AT III, concentration can be used as a guide replacement and heparin therapy for DIC.
- A low AT III concentration is a predictor of DIC.
- An elevated serum concentration of AT III may be found in any inflammatory process.

Therapy for DIC

DIC and hypercoagulation represent the result of a complex interaction between many factors. As such, the treatment of hypercoagulation and DIC represents a multi-faceted approach aimed at ensuring adequate oxygen tension in capillary beds, management of the underlying cause, replacement of pro-coagulants and anti-coagulants, and support of the target organs of thrombosis, particularly the liver, gastrointestinal tract, cardiac muscle, pulmonary parenchyma, kidneys, and central nervous tissue. These treatment goals are discussed briefly below.

1. Provide adequate blood flow and oxygen delivery to capillary beds – this is usually achieved with appropriate fluid therapy. The fluid therapy of choice varies depending on the clinical setting in which hypercoagulation has occurred. If the patient is in shock; immediate blood volume resuscitation with a combination of lactated Ringer's solution and a synthetic colloid such as dextran 70; or a combination of hypertonic saline and dextran 70 is preferred, as these fluids will minimize the volume of fluid required for intravascular volume expansion, and will minimize the extravasation of fluid from the intravascular to the extravascular space. If the patient has an active bleeding tendency, as determined by clotting tests, fresh frozen plasma (or whole blood if appropriate) is given at a rate of 10-20 ml/kg/12 hrs, following intravascular volume resuscitation, in order to prevent further bleeding into capillary beds. Maintenance of blood flow in capillary beds is achieved with a combination of crystalloids, synthetic colloids, fresh frozen plasma and whole blood. It is essential to monitor parameters such as the PCV, total protein level, albumin, ACT, and electrolytes to ensure the correct fluid is used in each patient. Despite seemingly adequate fluid resuscitation, some patients appear to remain in a hypotensive state, or appear to respond poorly to fluid therapy, i.e., they still

show signs of poor pulses, poor organ function – low urine output, vomiting, nausea, mental depression etc. These patients usually have sustained hypotension due to loss of arteriolar vascular tone that occurs in DIC and conditions of tissue hypoxia. These patients should have their blood pressure monitored, and a urinary catheter inserted, to determine if they are hypotensive or not. If hypotension is persistent despite adequate fluid resuscitation, low doses of dobutamine can be administered at a rate of 2-5 ug/kg/min in an effort to increase vascular tone. Mannitol and furosemide can be administered to patients with a combination of an elevated blood pressure and poor urine output.

2. Management of the underlying cause is essential to correcting the hypercoagulable state. For example, in sepsis, antibiotic therapy, fluid therapy, colloid therapy, and plasma/blood transfusions are the cornerstones of therapy. Removal of neoplastic lesions or chemotherapy is essential in patients with neoplasia, and immune-suppressive therapy in patients with hemolytic anemia.

3. Replacement of procoagulants and anti-coagulants is achieved with fresh frozen plasma. Prior to fresh frozen plasma administration, antithrombin III should be activated by incubating the bag of plasma with unfractionated heparin at 100 U/kg for 30 minutes. Without heparin activation of the anti-coagulant antithrombin III, the provision of pro-coagulant clotting factors in the plasma transfusion could potentiate additional intravascular thrombosis. Heparin should be continued for at least 36 hrs at a dose of 80 units/kg sc q 8 hrs.

4. Support of the end organs – the targets of thrombosis – is best achieved by adequate fluid resuscitation, provision of positive inotropic support when indicated, provision of clotting factors, and patient monitoring. In addition, specific therapy for organs such as the gut (ranitidine, metoclopramide, micro-enteral nutrition), kidneys (ensure adequate urine output and systolic blood pressure), lungs (oxygen supplementation, ventilatory support) and cardiovascular system (fluid therapy, positive inotropism, anti-arrhythmic therapy) is used.

Treatment of Coagulation Disorders

Treatment and potential therapies for patients with bleeding disorders are summarized in the following table.

Potential Therapies for the Patient with Hypocoagulation

Primary Hemostasis	
Thrombocytopenia • Immune-mediated	• Prednisolone 2-4 mg/kg/day • Vincristine 0.02 mg/kg IV once • Azothiaprine 2 mg/kg PO x 7 days, then EOD • Cyclosporine, cyclophosphamide • Doxycycline 5-10 mg/kg q 12-24 hrs
• Infectious	• Avoid NSAID's
Thrombocytopathia	• DDAVP (von Willebrand's Disease) • Cryoprecipitate (von Willebrand's Disease)
Secondary Hemostasis	
Anticoagulant rodenticide toxicity	• Vitamin K 2.5-5 mg/kg/day • Plasma – fresh frozen 10-20 ml/kg
Disseminated intravascular coagulopathy	• Treat underlying cause • Heparin – see later • Plasma – fresh frozen 10-15 ml/kg q 12 hrs
Liver disease	• Intravascular fluid support • Vitamin K 1-5 mg/kg q 24 hrs • Plasma – fresh frozen 10-15 ml/kg

Hypercoagulation: Pharmacological Manipulation

The treatment and prevention of thromboembolism should address all aspects of Virchow's triad; and includes minimizing vascular stasis through the maintenance of adequate tissue perfusion, and the prevention of prolonged immobility; minimizing vascular injury through the appropriate use and handling of intravenous catheters; and altering the hemostatic system through the appropriate use of drugs.

Pharmacological agents that inhibit hemostasis are classified as antiplatelet drugs, anticoagulants, and fibrinolysins. In general, anticoagulants and antiplatelet drugs do not lyse existing thrombi, but they may help to inhibit their propagation, and prevent recurrent thrombosis.

Antiplatelet Drugs

Indications

- Prevention of arterial thrombosis associated with cardiac disease, arteriosclerosis, and glomerulonephropathies.

- Management of Pulmonary Thromboembolism (PTE) – PTE in humans is associated with deep vein thrombosis; in veterinary medicine, PTE is seen secondary to numerous hypercoagulable states.

Cyclo-oxygenase inhibitors: Aspirin (acetylsalicylic acid)

Aspirin irreversibly inhibits platelet aggregation by acetylation of the enzyme cyclo-oxygenase, preventing the formation of prostaglandins G_2, H_2, and thromboxane A_2 (TxA_2) by platelets. This reaction is irreversible because platelets are anucleate, and unable to produce additional cyclo-oxygenase. When a dose of aspirin is given, all the platelets in circulation are affected for their entire lifespan. Vascular endothelial cells also produce cyclo-oxygenase, leading to prostacycline (PGI_2) production. Prostacycline is a potent inhibitor of platelet aggregation. Aspirin, therefore, produces a decrease in prostacycline production. The effect on endothelial cells, and hence prostacycline production is reversible, as the cells are nucleate, and are able to produce additional cyclo-oxygenase. Effective antiplatelet therapy with aspirin therefore depends on using a low dose that will affect platelets irreversibly, but allow vascular endothelial cells to recover, and resume prostacycline production.

Dose: dog 0.5 mg/kg PO q 12 hrs; cat 25 mg/kg PO twice weekly. The effect on prostacycline in cats is unknown at this dose. It is possible that ultra-low doses given more frequently will prove to be superior. The anti-platelet effect of aspirin persists for 7-10 days after cessation of therapy, corresponding to the lifespan of the platelet.
Aspirin has been shown to significantly reduce the incidence of thrombosis in humans with arteriosclerosis, myocardial infarction, ischemic stroke, and peripheral arterial occlusive disease by up to 40%. However, it is a relatively weak antiplatelet drug. It only slightly attenuates platelet aggregability responses to ADP and collagen, and does not inhibit ADP or platelet-activating factor (PAF) induced aggregability. The incidence of thromboembolic disease in patients on aspirin therapy remains substantial.

Calcium channel blockers have been shown to inhibit platelet aggregation induced by mediators such as ADP, collagen, and epinephrine. A study of feline hypertrophic cardiomyopathy involving therapy with diltiazem alone, or in combination with aspirin, found no significant difference in the incidence of thromboembolism.

Phosphodiesterase inhibitors increase concentrations of cAMP in platelets, thus inhibiting platelet calcium mobilization and adhesion molecule expression. Amrinone has been shown to inhibit platelet activation and to protect against experimental coronary artery thrombosis in dogs.

Thromboxane Synthetase Inhibitors such as dazoxiben prevent the formation of TxA_2 in platelets, but does not affect the endothelial production of prostacycline. Anti-aggregatory effects are weaker than those of aspirin are.

Anticoagulants

Anticoagulants inhibit the coagulation system either by enhancing natural anticoagulants, or by decreasing coagulation factor production. Anticoagulants are most useful in treating and preventing venous thrombosis.

Unfractionated Heparin

Unfractionated heparin is composed of naturally occurring mucopolysaccharides of varying molecular weights, and is bovine or porcine in origin. The primary mechanism of action of heparin is the potentiation of antithrombin III (ATIII) activity, leading to the inhibition of factor Xa and thrombin. Heparin also catalyses the inactivation of thrombin by plasma/heparin cofactor II. Heparin decreases blood viscosity, and improves blood flow by imparting negative charges to blood cells and vascular endothelium. Heparin increases vascular permeability, and enhances fibrinolysis by increasing levels of tissue plasminogen activator (t-PA).

The anticoagulant effects of a standard dose of heparin vary widely due to the following reasons:

- Varying absorption from injection sites.
- Plasma clearance depends on a dose-related renal clearance, and a non-dose-related saturable cellular mechanism.
- Heparin binds to plasma proteins, endothelium and platelets.
- There is a diurnal variation in PTT response in patients on heparin therapy in humans. It is unknown whether this occurs in dogs and cats.

The therapeutic range of heparin has been based on animal studies and subgroup analysis in humans. Prevention of growth of thrombi requires doses of heparin that prolong the PTT to twice that of normal. These doses are equivalent to a heparin concentration of 0.2 U/ml. Therefore; successful heparin therapy necessitates the monitoring of the PTT and heparin concentration. There is significant variation in PTT using different coagulometers, and it is recommended to establish a curve for each machine, based on PTT and associated heparin concentrations for 20-30 patients; thereafter, monitoring heparin concentrations is considered unnecessary.

Published doses of heparin in veterinary literature vary, and are largely based on anecdotal evidence. It would appear prudent to begin with protocols established in human medicine, and in animal models, and to monitor the PTT values closely. A suggested dose regime in patients is outlined as follows:

- *An intravenous bolus of 80 U/kg, followed by a CRI of 18 U/kg/hr. A PTT is evaluated 6 hours after bolus and initiation of CRI. Adjustments are made to the dosage using the table below.*
- *For prevention of thromboembolism using a subcutaneous low dose heparin, 100 U/kg q 8-12 hours may be used.*

Side effects of heparin therapy are generally limited to bleeding. Protamine sulphate may be given at a dose of 1 mg per 100 units of heparin, via slow intravenous injection. If 1 hour has elapsed since the last heparin dose, 50% of this dose is given; if 2 hours have elapsed since the last heparin dose, 25% of this dose is given.
While heparin may decrease ATIII concentrations, low levels of ATIII are more commonly related to the underlying disease process, e.g., sepsis, nephrotic syndrome etc.

Dosage adjustment for heparin therapy

PTT	Dose Change U/kg/hr	Additional action	Next PTT
< 1.2 x normal	+ 4	Re-bolus with 80 U/kg	6 hrs
1.2-1.5 x normal	+ 2	Re-bolus with 40 U/kg	6 hrs
1.5-2.3 x normal	0	0	6 hrs for first 24 hrs, then daily
2.3-3.0 x normal	-2	0	6 hrs
> 3.0 x normal	-3	Stop infusion for 1 hr	6 hrs

Low Molecular-weight Heparins

Low molecular weight heparins are manufactured from unfractionated heparin using chemical or enzymatic techniques. They have the following properties:

- Better bioavailability (90%) following subcutaneous injection than unfractionated heparin.
- Prolonged half-life and predictable clearance, enabling once or twice daily dosing.
- Predictable anti-thrombotic responses permitting treatment based on bodyweight rather than laboratory monitoring.
- Able to inhibit platelet bound factor Xa.
- Resistant to inhibition by platelet factor 4, large quantities of which are released by platelets in arterial thrombi.
- Less inhibition of platelet function and vascular permeability than unfractionated heparin, leading to lower incidence of hemorrhagic side effects.
- Have superior efficacy in the treatment and prevention of venous thrombosis.
- Efficacious and safe doses have not yet been established for dogs and cats.

Warfarin

Warfarin is a vitamin K antagonist that results in a decrease in availability of vitamin K1, making the carboxylation of vitamin K-dependant clotting factors II, VII, IX, X, and protein C impossible. The anticoagulant effect of warfarin is not immediate. Factor VII and protein C have the shortest half-lives (6-7 hrs). Inhibition of protein C is pro-thrombic, and this may lead to thrombosis in the first 24 hours of therapy, before factors II, IX, and X can be inhibited. It is recommended that heparin therapy overlap warfarin therapy in the first 24-48 hr of warfarin therapy. The major complication of warfarin therapy is hemorrhage.

Antithrombin agents such as hirudin, hirulog and argroban inhibit soluble and bound thrombin, are not inactivated by platelet factor 4, and have predictable bioavailability.

Factor Xa inhibitors are the focus of pre-clinical trials.

Fibrinolysins

Fibrinolysins are effective to some degree in almost all instances of thrombosis therapy. Their efficacy depends on the age of the thrombus, the fibrin content of the thrombus, plasminogen content, the location of the thrombus (arterial vs. venous). Recent and arterial thrombi lyse more readily. Systemic fibrinolytic states are the major complication with therapy, and are not uncommon. Additionally, reperfusion injury and hyperkalemia are common complications. Contraindications for thrombolytic therapy include active internal bleeding, hypertension, recent surgery, organ biopsy, and gastrointestinal bleeding.

Streptokinase

Streptokinase is isolated from the broth of beta-hemolytic streptococcal cultures. It binds circulating plasminogen and activates it. In the human and cat, the streptokinase-plasmin complex can activate the systemic conversion of plasminogen to plasmin and induces a systemic fibrinolytic state. In the dog, the streptokinase-plasmin complex is inactivated by alpha$_2$-antiplasmin, making streptokinase more thrombus-specific in the dog. In cats, results of studies show that approximately 33% of treated cats with arterial thromboembolism had a return of motor function when treated with streptokinase, however, a large number of these cats died because of hyperkalemia or systemic bleeding.

Urokinase and Pro-Urokinase

Urokinase is isolated from the urine. It is a naturally occurring plasminogen activator, converting plasminogen directly into plasmin. Urokinase is not clot-specific and can induce a systemic fibrinolytic state. Pro-Urokinase is clot specific, as they are not activated until they are absorbed onto fibrin.

Tissue-Type Plasminogen Activator (t-PA)

Tissue plasminogen activator is the major physiological activator of plasminogen. It has a low affinity for circulating plasminogen, and a high affinity for fibrin, and plasminogen activation occurs on the fibrin's surface. As the clot lyses, plasmin remains bound to the clot. Free plasmin is rapidly bound by alpha$_2$-antiplasmin. Tissue plasminogen activator is more efficacious than streptokinase and urokinase in providing thrombolysis, and is more effective in lysing aged thrombi. A study showing rapid thrombolysis in cats and a return of limb function of 43% within 2 days, approximately 50% of the treated cats died suddenly due to hyperkalemia, heart failure, or cardiac embolization. As success rates for return of limb function are similar with aspiring therapy, the risk to benefit.

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