Disorders of Coagulation - Part 1
Hemostasis

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 principle 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 serotonin, 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 A₂ 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 K₁ 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 is 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 table below:

<table>
<thead>
<tr>
<th>Down-regulator of clotting</th>
<th>Pro-thrombotic target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin</td>
<td>Factors Xa, XIIa, Xla, thrombin</td>
</tr>
<tr>
<td>Alpha-1 protease inhibitor</td>
<td>Factor Xla, elastase</td>
</tr>
<tr>
<td>Alpha-2 antiplasmin</td>
<td>Plasmin</td>
</tr>
<tr>
<td>Alpha-2 macroglobulin</td>
<td>Kallikrein, plasmin, thrombin</td>
</tr>
<tr>
<td>C1 inhibitor</td>
<td>Factor XIIa, kallikrein</td>
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<tr>
<td>Heparin cofactor II</td>
<td>Thrombin</td>
</tr>
<tr>
<td>Protein C</td>
<td>Factors VIIIa, Va</td>
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<tr>
<td>Tissue factor pathway inhibitor</td>
<td>Factor VIIa-tissue factor complex</td>
</tr>
</tbody>
</table>
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-Calciun 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 from a normal patient, 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 (thrombocytopenia).
Disorders of Coagulation - Part 2
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 $40 \times 10^9$ are required for clinical signs of spontaneous hemorrhage to occur, however counts of less than $100 \times 10^9$ 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 (thrombocytopenia)

The causes of primary hemostatic defects are summarized in the table below
Causes of Primary Coagulation Disorders in Dogs and Cats

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

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 reported clinical 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, activation of secondary hemostasis, and the formation of a fibrin plug will eventually cover the damaged blood vessel 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 = 100x10⁹/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 Willebrand's disease or severe thrombocytopenia or thrombocytopathia
- PT/APTT - normal; APTT may be prolonged in von Willebrand's disease, thrombocytopenia or thrombocytopathia
- CBC
  - Evidence of a regenerative anemia may be present 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 and 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) has 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

There are three goals in treating patients with thrombocytopenia

1. Stop the bleeding
2. Halt platelet destruction
3. Correct the underlying disorder

Further to these goals, there are adjunctive therapeutic measures that may be considered

1. Administration of fresh whole blood, and platelet rich transfusions is typically unrewarding.
2. Anticoagulant therapy in DIC (see later)
3. Gut protectants such as H₂ antagonists and anti-emetics may reduce nausea in some patients
4. Glucocorticoids are used in immune-mediated disease.
5. 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 $50 \times 10^9/l$
- 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.

- Azathiaprine – 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-dependent 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 10kg 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.
Thrombocytopenia

Etiology

The causes of thrombocytopenia/altered platelet function are listed in the table below.

<table>
<thead>
<tr>
<th>Inherited Thrombocytopenia</th>
<th>Disease-associated Thrombocytopenia</th>
<th>Drug-associated Thrombocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Von Willebrand's Disease</td>
<td>1. Anemia - alters blood viscosity</td>
<td>1. Antibiotics</td>
</tr>
<tr>
<td>a. Type I</td>
<td>2. Sepsis and DIC - alters platelet reactivity</td>
<td>a. Carbenicillin</td>
</tr>
<tr>
<td>b. Type II</td>
<td></td>
<td>b. Cephalothin</td>
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<tr>
<td>c. Type III</td>
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<td>c. Moxolactam</td>
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<tr>
<td>3. Liver disease</td>
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<td>d. Sulphonamides</td>
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<tr>
<td>5. Uremia - alters prostaglandin metabolism and reactivity of platelets</td>
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<td>b. Ibuprofen</td>
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<td></td>
<td></td>
<td>c. Phenylbutazone</td>
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<td></td>
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<td>d. Naproxen</td>
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<td>3. Cardiac/Respiratory</td>
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<tr>
<td></td>
<td></td>
<td>a. Aminophylline²</td>
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<td></td>
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<td>b. Isoproterenol</td>
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<td>c. Propanolol</td>
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<td></td>
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<td>d. Theophylline²</td>
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<td></td>
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<td>e. Verapamil ³</td>
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<td>4. Miscellaneous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a. Barbituates⁴</td>
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<tr>
<td></td>
<td></td>
<td>b. Dextran 70, pentaspan</td>
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<tr>
<td></td>
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<td>c. Heparin</td>
</tr>
</tbody>
</table>

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 Willebrand's Factor concentration.
- Determine drug history to rule out exposure to drug therapy that may result in thrombocytopenia
- 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

Thrombocytopenia - 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 multimers. The larger multimers are more effective in promoting platelet adhesiveness than smaller multimers
- 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 multimers 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 multimers are absent in these patients, which can result in severe bleeding
- German Wirehaired Pointer, German Shorthaired Pointer
**Type III von Willebrand’s Disease**
- All multimers 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 PG I₂, 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

**Thrombocytopenia - 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

**Thrombocytopenia - Hypercoagulation-Induced Thrombocytopenia and DIC**

These conditions will be reviewed under the heading “Tertiary Clotting Disorders”