DISORDERS OF PRIMARY HAEMOSTASIS

HAEMORRHAGE

- **haemorrhage = escape of blood from the cardiovascular system**

- a substantial tear in a blood vessel or a tear in a heart chamber → rapid escape of a substantial volume of blood = **haemorrhage by rhexis**

- escape of red blood cells one by one through minute or microscopically imperceptible defects in vessel walls = **haemorrhage by diapedesis**

Location of Haemorrhage

- **haemothorax** = haemorrhage into the pleural cavity
- **haemopericardium** = haemorrhage into the pericardial sac
- **haemoperitoneum** (**haemabdomen** or **haemoabdomen**) = haemorrhage into the peritoneal cavity
- **haemarthrosis** = haemorrhage into a synovial joint
- **haematuria** = haemorrhage into urine
- **haemoptysis** = coughing of blood
- **haematemesis** = vomiting of blood
- **haematochezia** = passage of fresh blood in faeces (typically over the surface of stools)
- **dysentery** = diarrhoea containing blood
- **melaena** = diffuse, dark red-black discolouration of faeces due to upper alimentary tract haemorrhage or swallowing of blood from the respiratory tract
- **epistaxis** = bleeding from the nose
- **hyphaema** = haemorrhage into the anterior chamber of the eye

Size of Haemorrhages

- **petechiae** = tiny, pinpoint foci of haemorrhage, 1-2 mm in diameter
  - petechiae are typically found in skin and on mucosal and serosal membranes
- **purpura** = slightly larger haemorrhages $\geq$ 3 mm in diameter

(NOTE – The term **purpura** is also used clinically to describe conditions in which there are extensive petechial, purpural and ecchymotic haemorrhages scattered over serosal and mucous membranes +/- skin.)

- **ecchymoses = larger foci of haemorrhage, 2-3 cm in diameter; usually blotchy and of** irregular shape

- **paintbrush (or suffuse or suffusive) haemorrhages = linear or streaky haemorrhages,** especially over serosal or mucosal membranes, as if a brush dipped in red paint has been hastily splashed across the tissues

- **petechiae, purpura, ecchymoses and paintbrush haemorrhages** are visible to the naked eye but are **not palpable**

- **haematoma = a palpable, discrete, space-occupying mass of clotted blood within tissues**
  - small haematomas are common at sites of difficult venipuncture

Age of Haemorrhage

- the gross colour of haemorrhage depends on whether the blood was arterial or venous, the volume of extravasated blood and the time elapsed since haemorrhage occurred
- extravasated blood cells and coagulated fibrin are removed by lysis and especially by phagocytosis
- erythrophagocytosis by macrophages commences within a few hours of onset of haemorrhage

- in the **acute phase**, bruises appear **red-blue** due to the presence of poorly oxygenated haemoglobin

- in the **subacute phase**, bruises appear **blue-green** due to the formation within macrophages of biliverdin and bilirubin (derived from the porphyrin component of haemoglobin)

- in the **chronic phase**, bruises appear **gold-brown** due to the formation of haemosiderin and, to a lesser extent, haematoidin +/- lipofuscin pigments

- **haemosiderin** (composed of ferritin micelles (iron + apoferritin) derived from the iron component of haemoglobin) first appears microscopically within macrophages by 24-48 hours after the onset of haemorrhage
- sufficient haemosiderin accumulation to cause gross yellow discolouration is not expected until 2-3 days after haemorrhage
- **haematoidin** is a bright golden extracellular pigment composed of precipitates of bilirubin complexed with tissue proteins
- **lipofuscin** is an intracellular yellow-brown pigment derived from peroxidation of phospholipids of membranes of damaged cells in the area

- from 5-7 days after onset, haematomas become enveloped by **granulation tissue**
- following phagocytosis of the extravasated blood, the cavity of a haematoma is ultimately filled in by **scar tissue** (fibrosis)

Clinical Significance of Haemorrhage

- the clinical significance of haemorrhage depends on its **location** and the **rate** and **volume** of blood loss

- most tissues can tolerate a degree of haemorrhage without loss of function
- however, bleeding that might be trivial in the subcutis may be fatal in the brain (**stroke** or **cerebrovascular accident**) or if extra-cerebral but **intra-cranial** (e.g. subdural haematoma) because of space limitations of the skull and increased intra-cranial pressure
- **intra-myocardial** haemorrhage may weaken or destroy regions of the myocardium or interfere with cardiac conduction pathways → disturbance in the cardiac rhythm
- rapid haemopericardium may → **cardiac tamponade** (impaired diastolic filling of the right ventricle → acute right-sided heart failure)
- haemorrhage into or beneath the **retina** may → permanent loss of vision

- **internal haemorrhage** (haemorrhage into tissues or body cavities) → reutilisation of plasma proteins and iron
- some extravasated erythrocytes may be resorbed into lymphatics and returned to circulation
- other extravasated erythrocytes will undergo lysis or phagocytosis but reutilisable constituents will be salvaged

- recurrent or prolonged **external haemorrhage** (e.g. from the skin or gastrointestinal, respiratory, urinary or reproductive tracts) → hypoproteinaemia and iron deficiency anaemia

- rapid removal of up to 20% of total blood volume or slow loss of larger volumes may have little impact on healthy animals
- loss of 20-40% of blood volume → **haemorrhagic (hypovolaemic) shock**
- the more rapid the blood loss, the smaller the volume of blood lost necessary to induce shock
- loss of 50% or more of blood volume → **death** without an immediate blood transfusion

Causes of Haemorrhage

- haemorrhage is a non-specific finding; an underlying cause needs to be identified

- the **most common cause of haemorrhage is <u>physical trauma</u>**
- **other causes** include:

  - severe tissue inflammation or ulceration of a mucous membrane with damage to adjacent/underlying blood vessels
  - spontaneous rupture of a well-vascularised organ (e.g. liver or spleen) or tumour (e.g. haemangiosarcoma) or invasion of a blood vessel by a malignant neoplasm
  - ectoparasites (e.g. fleas) and endoparasites (e.g. hookworms, coccidia)
  - inflammation, degeneration or necrosis of a blood vessel - e.g. vasculitis, renal failure, bacteraemia, viraemia, endotoxaemia
  - rupture of a weakened vessel wall - e.g. vascular aneurysm, copper deficiency, scurvy
  - active inflammation (hyperaemic phase) → haemorrhage by diapedesis
  - passive congestion → increased hydrostatic pressure in capillary beds → haemorrhage by

diapedesis
- inherited or acquired defects in primary and/or secondary haemostasis

THE HAEMORRHAGIC DIATHESES

- haemorrhagic diatheses are clinical disorders of haemostasis characterised by a bleeding tendency
- haemorrhage may occur spontaneously or there may be excessive bleeding in response to minor trauma

- the haemorrhagic diatheses can be broadly subdivided into:

Disorders of Primary Haemostasis

- platelet deficiency (**thrombocytopenia**)
- platelet dysfunction (**thrombocytopathy**, **thrombopathy** or **thrombopathia**)
- **von Willebrand's disease** (deficiency of von Willebrand factor)
- **damage to small blood vessels**

Disorders of Secondary Haemostasis (Lecture 17)

- **inherited deficiency of one or more coagulation factors**
- decreased coagulation factor activity due to **vitamin K antagonism** or **deficiency**
- decreased coagulation factor synthesis due to **severe acute** or **chronic hepatic parenchymal disease**
- **excessive fibrinolysis** or **fibrinogenolysis**

Combined Disorders of Both Primary and Secondary Haemostasis

- **disseminated intravascular coagulation** (DIC) (Lecture 29)

LOCALISING THE HAEMOSTATIC DEFECT

- clinical signs may provide an early clue as to whether bleeding is referable to a defect in primary or secondary haemostasis or both (**Table 1**)

- because the primary haemostatic platelet plug is short-lived and ultimately enveloped by fibrin, **disorders of primary haemostasis** provoke multiple short-lived bleeds that cease once the coagulation cascade generates fibrin
- bleeding commences immediately after venipuncture or other trauma and is prolonged for a few minutes but is ultimately terminated by formation of fibrin
- multiple, small volume, superficial, petechial, purpural, ecchymotic and/or paintbrush haemorrhages are typical over the skin, mucous membranes and serosal surfaces
- epistaxis is common and bleeding from other mucous membranes may cause haematuria, haematemesis, melaena and/or haematochezia
- haematomas may develop but are more commonly seen in animals with disorders of

secondary haemostasis

- animals with **disorders of secondary haemostasis** can form short-lived, primary haemostatic platelet plugs but cannot generate (or, rarely, maintain) the fibrin plug
- bleeding after venipuncture or other trauma is usually delayed by the platelet plug but, once bleeding commences, it may be prolonged and severe
- deep haematomas are common
- severe haemorrhage into body cavities, joints and skeletal muscles may occur
- haemorrhage from mucous membranes (e.g. melaena, epistaxis) occurs occasionally

Table 1 – Clinical Clues in Haemostatic Disorders

Defect in Primary Haemostasis	*Defect in Secondary Haemostasis*
Bleeding immediately after venipuncture	Delayed bleeding after venipuncture
Small volume bleeds	Large volume bleeds
Usually bleeding from multiple sites	May bleed from multiple sites but often localised to one site
Petechiae and ecchymoses common	Petechiae and ecchymoses rare
Haematomas uncommon	Haematomas common
Bleeding from mucous membranes, into skin and over serosal surfaces	Bleeding into muscles, joints and/or body cavities +/- Bleeding from mucous membranes

DISORDERS OF PRIMARY HAEMOSTASIS

THROMBOCYTOPENIA

- **thrombocytopenia is the most common acquired haemostatic disorder in dogs and cats**

- thrombocytopenia may reflect **decreased platelet production in the bone marrow** or **destruction**, **consumption** or **sequestration** of platelets
- **massive haemorrhage** (due to another cause) can also result in thrombocytopenia

Decreased Platelet Production

- this is the **most common mechanism of thrombocytopenia in cats** and is largely due to **retroviral infection** (especially feline leukaemia virus (FeLV)) and/or **myeloproliferative** or **lymphoproliferative disease**

- causes of decreased marrow production of platelets from megakaryocytes in domestic animals

include:

- **myelophthisis** - replacement of haemopoietic bone marrow by neoplastic tissue (e.g. leukaemia), collagen (myelofibrosis), bone (osteosclerosis) or inflammatory exudate (e.g. chronic granulomatous osteomyelitis in systemic fungal infections)

- **marrow aplasia or panhypoplasia** - due to injury to haemopoietic stem cells or their progeny and/or marrow stromal cells (with decreased production of growth stimulant factors or increased production of growth suppressor factors)
  - e.g. **drug cytotoxicity** - chemotherapeutic agents, chloramphenicol, sulphadiazine, oestrogen (dogs and ferrets), griseofulvin (cats), methimazole (cats)
  - e.g. **toxins** - aflatoxins, stachybotryotoxin, bracken fern, benzene
  - e.g. **ionising radiation**
  - e.g. **viral infection** - canine and feline parvovirus, FeLV, feline immunodeficiency virus (FIV), equine infectious anaemia (EIA) virus, bovine viral diarrhoea (BVD) virus
  - e.g. **other infectious agents** - late stages of infection with *Ehrlichia canis* and other rickettsial species

- **megakaryocytic hypoplasia** - a rare form of immune-mediated thrombocytopenia reported in dogs, with autoantibodies directed against megakaryocytes

- some of the above conditions will manifest first as neutropenia, followed by thrombocytopenia and finally progressive non-regenerative anaemia, in accordance with the lifespans of neutrophils, platelets and erythrocytes

Platelet Destruction

- platelet destruction is the **most common mechanism of thrombocytopenia in dogs**, with the destruction most often being **immune-mediated**

- **immune-mediated destruction**

  - in **primary immune-mediated thrombocytopenia** (= **autoimmune thrombocytopenia**), autoantibodies are produced against platelet antigens
  - megakaryocytes may also be targeted if the antibody is directed against a common membrane antigen
  - **common in dogs**

  - in **secondary immune-mediated thrombocytopenia**, destruction of platelets is secondary to another disease process (e.g. systemic lupus erythematosus (SLE), neoplasia (especially lymphoma), viral, bacterial or rickettsial infection, or drug administration (e.g. antibiotics such as penicillin and sulphonamides, NSAIDs such as aspirin, thiazide diuretics, and cardiovascular drugs such as digoxin)

- e.g. a mild, transient and usually subclinical thrombocytopenia (platelet count ≥ 100 x 10^9/L) may develop 3-10 days post-vaccination with **modified live virus vaccines** (e.g. canine distemper virus, canine parvovirus and feline parvovirus vaccines)
- e.g. intravenous **heparin** administration can cause a mild thrombocytopenia in **horses**

- **neonatal alloimune** (or **isoimmune**) **thrombocytopenia** occurs in piglets and has also been reported in foals
- immune-mediated destruction of platelets (and, later, in affected piglets, megakaryocytes in bone marrow) is due to receipt in colostrum of maternal antibodies directed against paternal epitopes on the surface of platelets

- **non-immune-mediated destruction**

  - **infectious agents** (e.g. *Anaplasma platys*, a rickettsial parasite that infects platelets of dogs; e.g. many viruses and bacteria may destroy platelets directly or by promoting phagocytosis of platelets by hyperactive tissue macrophages)

Consumption (Utilisation) of Platelets

- platelet consumption due to formation of numerous microthrombi is typical of **disseminated intravascular coagulation** (DIC) (Lecture 29)
- less severe consumption of platelets in formation of haemostatic plugs can also be a feature of conditions in which there is significant vascular endothelial injury:

  - e.g. heartworm (*Dirofilaria immitis*) infection
  - e.g. haemangiosarcoma
  - e.g. endotoxaemia
  - e.g. severe liver necrosis
  - e.g. vasculitis

Platelet Sequestration

- in health, 30-40% of all platelets are located in the spleen
- platelets may be sequestered in the spleen or liver when these organs are enlarged or when their microenvironment is altered (e.g. splenic/hepatic congestion or widespread malignancy)

- **hypothermia** can → sequestration of platelets within the liver

- **endotoxaemia** and other causes of **"shock lung"** → sequestration of platelets within pulmonary capillaries

- in most circumstances, sequestration causes only a **mild thrombocytopenia** that is **unlikely to cause overt bleeding**

Massive Acute Haemorrhage

- massive acute haemorrhage can cause a thrombocytopenia but the latter is usually **mild** (platelet count ≥ 100 x 10^9/L) and **subclinical** because of the large splenic platelet reservoir

THROMBOCYTOPATHIES

- platelet function disorders may be congenital or acquired

Inherited Platelet Dysfunction

- **rare** but reported in dogs, cats, cattle, horses and pigs
- e.g. **Glanzmann thrombasthenia** - otterhounds, Great Pyrenees dogs, Thoroughbred and other horse breeds
  - platelets are deficient in surface glycoprotein receptors for fibrinogen → defective platelet aggregation
- e.g. **Chédiak-Higashi syndrome** - Persian cats, Hereford cattle
  - platelets lack discernible dense granules and are deficient in adenine nucleotides, serotonin and divalent cations
- e.g. **platelet dense granule deficiency** - American cocker spaniels
- e.g. **inherited thrombopathia** - basset hounds, Spitz dogs, Simmental cattle
  - absence or dysfunction of a signal transduction protein necessary for platelet release of dense granules and exposure of binding sites for fibrinogen

Acquired Platelet Dysfunction

- acquired platelet function defects are probably quite common but go largely unrecognised because **spontaneous haemorrhage is uncommon**

- **drugs** known to affect platelet function include:

  - **cyclooxygenase (COX) inhibitors** - e.g. **aspirin** irreversibly acetylates COX in platelets and megakaryocytes → inhibition of thromboxane A_2 production → impaired platelet function for up to 5 days
    - e.g. **ibuprofen** and **other NSAIDs** reversibly inactivate COX → impaired platelet function for usually < 6 hours
  - **β-lactam antibiotics** - e.g. penicillin, cephalosporins
  - **halothane** and **barbiturate anaesthetics**
  - **phenothiazine tranquilisers**
  - **calcium channel blockers** - e.g. diltiazem, verapamil
  - **sodium channel blockers** - e.g. quinidine
  - **β-adrenergic blockers** - e.g. propanolol
  - **anti-histamines**

- **diseases** that can cause platelet dysfunction include:

  - **systemic lupus erythematosus**
  - **myeloproliferative diseases** - e.g. leukaemias
  - **retroviral infections in cats** - especially FeLV
  - **hyperglobulinaemia** - e.g. multiple myeloma, feline infectious peritonitis
  - **chronic liver disease**
  - **renal failure**
  - ***s*nake envenomation**

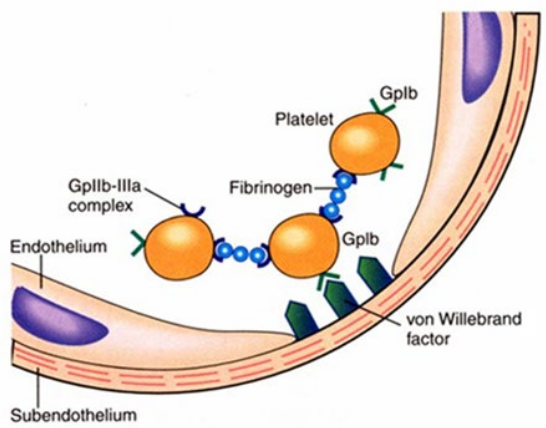
VON WILLEBRAND'S DISEASE

- von Willebrand's disease (vWD) is the **most common inherited bleeding disorder of dogs**
- reported in more than 50 dog breeds
- rare in cats (Himalayans), horses, cattle, pigs and rabbits

- vWD is caused by either an **absolute deficiency** or **decreased functional activity of von Willebrand factor** (vWf)

- vWf is a large glycoprotein that mediates the adhesion of platelets to exposed subendothelial collagen via their surface GpIb receptors (Figure 1)
- once tethered by vWf, platelets can become anchored to collagen by their GpIa/IIa receptors

Figure 1 – Platelet Adhesion to Subendothelial Collagen



Reference: "Robbins and Cotran Pathologic Basis of Disease" – V. Kumar, A.K. Abbas and N. Fausto. 7th edition, Saunders, Philadelphia, 2005

- in damaged blood vessels with low shear rates (e.g. capillaries), platelet adhesion to the collagen is mediated by other platelet membrane glycoprotein receptors (GpIa/IIa and GpVI)
- **in damaged blood vessels with high shear rates** (e.g. arteries and arterioles), **vWf is essential for platelet adhesion to collagen** (i.e. without vWf, the platelets will be dislodged by blood flow)
- vWf also contributes to platelet aggregation

- vWf is expressed by subendothelial connective tissues
- vWf is also synthesised by vascular endothelial cells and either stored within them or secreted into plasma
- injured endothelium releases stored vWf so that it can bind locally to exposed collagen
- megakaryocytes also synthesise vWf and, in some species (e.g. humans and cats but NOT dogs), circulating platelets also contain significant quantities of vWf (within alpha granules)

- within plasma, vWf circulates as a complex (FVIII:C) with clotting factor VIII which it stabilises and protects from degradation by proteases

- vWf is a heterogeneous glycoprotein composed of small, medium and large multimers of 270 kDa polypeptide subunits linked to each other by disulphide bonds
- the largest multimers are most active in primary haemostasis

- there are **three forms of inherited vWD** that differ in the severity of clinical bleeding
- all three forms have an **autosomal recessive pattern of inheritance** in **dogs**

Type 1 vWD

- the **most common form of vWD in dogs** (> 90% of cases)
- all of the various multimer chains of vWf are present but in reduced plasma concentrations (< 50% of normal)
- e.g. **doberman pinscher** (in the not so distant past, approximately 60% of dobermans in Australia were thought to be carriers of the trait), Welsh corgis, German shepherd dogs, golden retrievers, and poodles
- may be **subclinical** or cause **mild to moderate bleeding**
- bleeding does not usually occur until the plasma vWf concentration falls below 20% of normal

Type 2 vWD

- decreased plasma vWf concentration is associated with a disproportionate loss of the large high m.w. multimers
- can be responsible for **severe haemorrhage**
- rare but reported in German short-haired and wire-haired pointers
- also reported in horses

Type 3 vWD

- there is virtually negligible vWf in plasma, with all multimers absent
- **Scottish terriers** (the main breed affected in Australia), Shetland sheepdogs, Chesapeake Bay retrievers and Dutch kooikers
- trauma in homozygotes → **severe haemorrhage**

Acquired vWD

- an **acquired form of vWD** has been demonstrated in dogs in conditions of high shear forces within the circulation (e.g. severe subaortic stenosis)
- blood turbulence → unfolding of plasma vWf and immediate cleavage by a metalloprotease into smaller, less functional multimers

Clinical Signs

- **dogs with vWD usually do not bleed spontaneously**
- instead, excessive haemorrhage is triggered by hair clipping, venipuncture or other trauma
- perinatal mortality (including abortions and stillbirths) may be high in affected litters of puppies (especially with Type 2 or 3 vWD)
- excessive bleeding may be observed during tooth eruption or oestrus
- if spontaneous haemorrhage does occur, it is often as **diffuse oropharyngeal bleeding**
- epistaxis, gastrointestinal bleeding and/or haematuria may also occur
- **petechiae are infrequently seen in dogs with vWD**
- if there is a concurrent reduction in plasma factor VIII:C activity, **affected dogs may develop haematomas**

- **horses** with type 2 vWD may have haemarthrosis and/or haematomas

Treatment

- a fresh or fresh frozen plasma transfusion or cryoprecipitate can provide adequate vWf and FVIII:C to traumatised vWD patients
- administration of **desmopressin** (synthetic vasopressin) (DDAVP (1-deamino-8-D-arginine-vasopressin)) 30-90 minutes prior to surgery can boost the plasma concentration of vWf in dogs with Type 1 vWD for several hours, by promoting release of stored vWf from endothelial cells

DAMAGE TO SMALL BLOOD VESSELS

- diseases that damage small blood vessels can be responsible for spontaneous haemorrhage
- bleeding is typically of primary haemostatic type (i.e. petechiae, purpura, ecchymoses and paintbrush haemorrhages) but may be overshadowed by other clinical signs

- **causes** include:

  - **toxaemia** - e.g. bacterial endotoxins or exotoxins
  - **bacteraemia**
  - **endotheliotropic viral infections** - e.g. canine adenovirus-1, canine herpesvirus
    - e.g. hog cholera, African swine fever
  - **uraemia** (renal failure) - due to various circulating metabolic waste products
  - **infectious vasculitis** - e.g. equine herpesvirus-1, equine viral arteritis
    - e.g. Rocky Mountain spotted fever (*Rickettsia rickettsii*) in dogs
  - **immune-mediated vasculitis** - e.g. feline infectious peritonitis virus
    - e.g. bluetongue virus
    - e.g. malignant catarrhal fever
    - e.g. systemic lupus erythematosus
    - e.g. drug-induced vasculitis
  - **fragility of blood vessels** - fragility may be caused by decreased or defective collagen in vessel walls; decreased platelet responsiveness to abnormal collagen may also contribute to haemorrhage
    - e.g. **vitamin C deficiency** (**scurvy**) in guineapigs, pigs and primates
    - e.g. **inherited collagen dysplasia syndromes** in ruminants, horses, dogs, cats and rabbits (e.g. Ehlers-Danlos syndrome or cutaneous asthenia, associated with skin hyperelasticity)
    - e.g. **skin fragility syndromes** in **cats** caused by **hyperadrenocorticism** or **diabetes mellitus**

- assessment of blood vessel integrity and diagnosis of diseases such as vasculitis, inherited collagen disorders or skin fragility syndrome require histopathology
- collection of biopsy samples may be problematical in a bleeding patient and so is not usually performed until all other possible causes of haemorrhage have been excluded