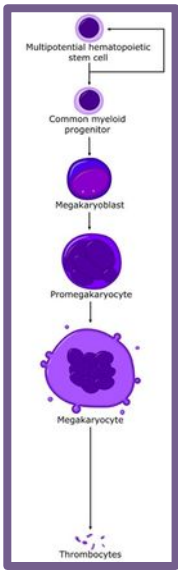
Essential Thrombocythemia and Reactive Thrombocytosis



Thrombopoiesis: a brief review

- ↑ PLT count/above reference interval established for species
- Derived from megakaryocytes in bone marrow
  - Fragmentation of cytoplasmic extensions→proplatelets
  - Requires adhesion of megakaryocyte to subendothelial matrix components, cytoskeletal elements, pro-apoptotic enzymes, matrix metalloproteinases, and shear forces
- Stimulated by unknown mechanism
  - Megakaryocyte production mediated by thrombopoietin
    - Acts as a differentiation factor-stimulates stem cells to differentiate along the megakaryocytic lineage
    - Prevents apoptosis
  - Platelet release involves stromal cell-derived factor-1

Methods of platelet enumeration

- Stained peripheral blood smear
- Manual hemocytometer
- Quantitative buffy coat analysis
- Impedence/laser-induced light scatter

Causes of thrombocytosis

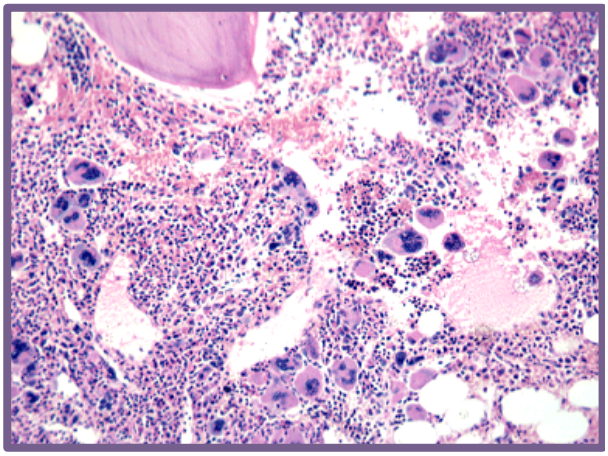
- **Pseudothrombocytosis**
  - Erroneous inclusion of RBC/WBC fragments or cellular debris into platelet enumeration
  - Estimated counts should be verified by stained blood smear evaluation
- **Physiologic thrombocytosis**
  - Release of platelets from splenic red pulp
  - Mediated by epinephrine
- **Drug-induced thrombocytosis**
  - Associated w/ vincristine and epinephrine
  - Data for glucocorticoids are conflicting
- **Reactive thrombocytosis**
  - Cytokine stimulation of thrombopoiesis
  - Secondary to various disorders→
  - Most commonly w/ cancer or inflamm
  - Mediated by a variety of cytokines:
    - TPO, IL-6, GM-CSF, IL-3, IL-11 and EPO
    - IL-6 is thought to act by induction of TPO production in hepatocytes
- **Essential thromobocythemia**
  - Chronic myeloproliferative disease
  - Clinical signs-not common
    - Microvascular thrombosis
      - Typically in arteries supplying brain, heart, and extremities
      - Mechanism unknown
    - Hemorrhage is associated with very high platelet numbers
      - Typically in the skin or mucosa
      - Attributed to type II vWdz
  - Diagnosis
    - Marked thrombocytosis w/ megakaryotcyte hyperplasia, leukocyte and erythrocyte counts generally w/in normal limits
    - Giant hypoerlobulated megakaryocytes diffuse or in small loose clusters with normo to slightly hypercellular marrow
    - Difficult to differentiate from reactive thrombocytosis
  - Prognosis:
    - Indolent dz w/ affected patients living fairly normal lifespans
    - Rarely Δ 's to leukemia, can Δ to chronic idiopathic myelofibrosis
  - Tx pursued if thrombohemorrhagic dz contributes to pt morbidity
    - Platelet apheresis, hydroxyurea, anagrelide, interferon-α
  - Only 2 case reports of essential thromobocythemia in vet species
- **Familial/Inherited thrombocytosis**
  - Results in high platelet counts observed at birth or very early age
  - Asymptomatic or thrombohemorrhagic

TABLE 80.2 Causes and Associated Mechanisms of Thrombocytosis[23,51,54,56]

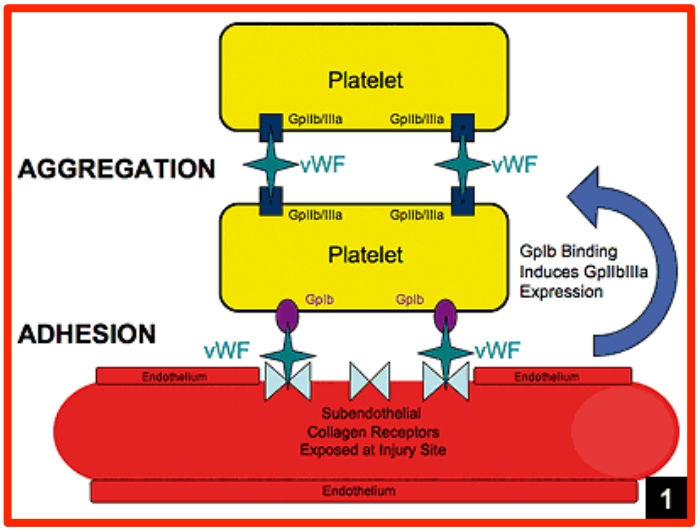
Cause	Mechanism
Pseudothrombocytosis	
Red cell ghosts or fragments, fragile leukocytes	Non-platelet cell fragments are counted as platelets
Physiologic	
Epinephrine (trauma, exercise, excitement)	Splenic contraction
Post-splenectomy	Lack of sequestration
Drug-induced	
Epinephrine	Splenic contraction
Vincristine	Enhanced megakaryopoiesis
Reactive	
Inflammation, infection, neoplasia (hematopoietic and non-hematopoietic), trauma, rebound from thrombocytopenia	Cytokine-mediated (directly or indirectly through TPO production) enhanced megakaryopoiesis
Iron deficiency-related	Unknown
Neoplasia involving megakaryocytes	
Chronic myeloproliferative disease: essential thrombocythemia, chronic myeloid leukemia, chronic basophilic leukemia, polycythemia vera, chronic idiopathic myelofibrosis	Acquired or inherited genetic mutations causing TPO-independent proliferation, e.g. JAK2 signaling mutations
Acute megakaryocytic leukemia	Acquired or inherited genetic mutations
Hereditary/familial[a]	Inherited genetic mutations

TABLE 80.4 Criteria for Differentiating Between Reactive Thrombocytosis and Essential Thrombocythemia in Humans[52,63]

Criteria	Reactive Thrombocytosis	Essential Thrombocythemia
Clinical		
Underlying disease	Yes	No
Thrombosis or hemorrhage	No[a]	Yes
Splenomegaly	No	Mild to moderate (due to extramedullary hematopoiesis)
Laboratory		
Platelet morphology	Normal	Normal, may see hypogranular/giant platelets
Bone marrow[b]	Normo- to hypercellular (concurrent granulocytic hyperplasia) Megakaryocytic hyperplasia (normal size, morphology)	Normocellular megakaryocytic hyperplasia (large cells, hyperlobulated or "staghorn" nuclei seen in loose clusters)[b]
Inflammatory markers (IL-6, fibrinogen, C-reactive protein)	High (may be normal)	Normal (may be high)
TPO levels	High or normal	High or normal[c]
Platelet aggregation	Normal	Abnormal (particularly to epinephrine) in 63–100%



von Willebrand Disease



Disease mechanism

- Quantitative and functional deficiency in vWf
- vWf physiology
  - Primarily synthesized and stored in the endothelial cells
  - Circulates as linear strings of subunits assembled w/in the endoplasmic reticulum and golgi apparatus
  - Two subunits form a dimer, dimers combine to multimers
  - vWf acts as a carrier for coagulation factor VIII
- Following injury:
  - vWf binds to subendothelial collagen
  - Conformation change→interaction w/ platelet GP 1b
  - Facilitates interplatelet bridging by platelet GP IIbIIIa
  - vWf deficiency leads to failure of platelet plug assembly, particularly under high shear conditions

Disease classification/ subtypes

- **Type 1**: partial quantitative deficiency, full complement of functional multimers
  - Clinical severity correlated to degree of reduction in [vWf]
  - Most common type in dogs
- **Type 2**: two variants
  - 2A: preferential loss of ↑ MW multimers, ↓ [plasma vWf]
    - Drastic reduction in platelet adhesion
  - 2B: defect that enhances binding affinity of platelet vWf receptor
- **Type 3**: complete absence of plasma vWf, concomitant ↓ in FVIII (typically mild in dogs)

TABLE 81.1 Classification of von Willebrand Disease

Type	VWF Defect	Affected Species (Breeds)
1	Partial quantitative deficiency, residual VWF has normal structure and function	Dog (Airedale, Akita, Bernese mountain dog, Dachshund, Doberman pinscher, German shepherd, Golden retriever, Greyhound, Irish wolfhound, Kerry blue terrier, Manchester terrier, Miniature pinscher, Papillon, Pembroke Welsh Corgi, Poodles, Schnauzer, other purebreeds and mixed breed dogs) Horse (Arabian) Mouse (RIIIS/J)
2A	Selective loss of large VWF multimers, decreased VWF-platelet & collagen interactions	Dog (German shorthaired pointer, German wirehaired pointer) Cow (Simmental) Horse (Quarter Horse, Thoroughbred)
2B	Increased VWF affinity for platelet glycoprotein Ib	No animal cases
2M	Impaired VWF binding to platelet glycoprotein Ib, normal multimer structure	No animal cases
2N	Decreased VWF binding to factor VIII	No animal cases
3	Complete VWF deficiency	Dog (Dutch Kooiker, Scottish terrier, Shetland sheepdog; sporadic cases Border collie, Chesapeake Bay retriever, Cocker spaniel, Eskimo dog, Labrador retriever, Maltese, Pitbull, and mixed breed) Cat (Himalayan)

von Willebrand factor assays

- **Quantitative**
  - vWf ag
    - Immunologic quantitation of [vWf]
    - ELISA or latex immunoassay
    - Reported as a percentage of normal
    - Anything ↓ 50% is considered deficient
- **Functional and structural**
  - Measure aspects of vWf interaction w/ platelets, collagen or FVIII
  - Ristocetin cofactor assay measures vWf dependent platelet agglutination
  - Collagen binding assays have replaced ristocetin cofactor assays
    - More readily standardized
    - Considered initial screening to detect type 2 vW dz
- **Point-of-care**
  - BMBT will be prolonged w/ severe forms of vWdz
    - Also influenced by platelet count, hematocrit and other conditions altering blood viscosity and platelet function
  - Platelet function analyzer (PFA-100) measures plug formation in whole blood under high shear flow
  - Closure time refers to the time necessary to occlude an aperture coated with collagen and ADP or epinepherine

Acquired vWD

- Ab-mediated clearance
- Proteolysis w/ shear injury in heart dz
- ↑ platelet binding in thrombotic and neoplastic dz
- Hypothyroidism
- HES/dextran administration

Hereditary vWD

- Autosomal, typically recessive trait in type 2 and 3, dominant in type 1
- Homozygotes inherit mutant vWf from both parents, invariable express a bleeding tendency
- Heterozygotes have 1 normal and 1 mutant vWf allele and are clinically normal
- In type 1, incomplete penetrance → variability of disease expression b/t individuals and variable clinical severity

Clinical management of vWD

- **Clinical diagnosis**
  - Clinical signs
    - Mucosal hemorrhage, cutaneous bleeding
    - Prolonged bleeding following surgery or trauma
    - Bleeding tendency ranges from mild to severe w/ type 1, typically will only have excess bleeding w/ trauma or sx

    - Types 2 and 3 are invariably severe in bleeding disorders
- Diagnostic evaluation
    - Routine screening performed on patients w/ bleeding tendencies
    - First perform platelet enumeration and coagulation panel testing
    - Then point-of-care tests of primary hemostasis and/or vWf:Ag performed
    - Prolonged point-of-care tests alongside vWf:Ag<50% consistent with vWf deficiency
    - vWf:Ag<0.1% is definitive for type 3 vWdz
    - Diagnosis of type 2 based on a quantitative deficiency alongside structural and functional abnormalities
        - A disproportionate defect of collagen binding is diagnosed as a vWf:Ag/vWf:CB > 2
- **Treatment**
    - Transfusion therapy
        - Early, high-dose transfusion to rapidly increase plasma vWf
        - Patients w/ type 2 and 3 vWdz invariably require transfusion to undergo invasive procedures or stop active bleeding
        - Cryoprecipitate best component for rapid vWf replacement (1 unit/10kg, w/ unit derived from a 200 mL unit of FFP)
        - FFP is an acceptable alternative (10-15 mL/kg)
        - Plasma half-life of vWf is 12 hours, necessitating q8-12 hr transfusion for severe hemostatic stress
    - Non-transfusion therapy
        - Avoid invasive procedures
        - Cautery, topical tissue adhesive, multilayer closure, and pressure wraps
        - Avoidance of anti-platelet and anti-coagulant drugs
        - Correct conditions which may exacerbate bleeding tendency:
            - Thrombocytopenia, uremia, hyperproteinemia, anema, hypothyroidism, and liver disease
        - Desmopressin acetate stimulates endothelial V2 receptors→release of vWf intracellular stores
            - Used best as a preoperative prophylactic measure in type 1 dogs (1 ug/kg)
            - Repeat doses will decrease efficacy (tachyphylaxis)

Questions

1. Desmopressin acetate is utilized in von Willebrand disease as it
    a. Functions to release platelets from the bone marrow by endothelial vasopressin receptor mediated enhancement of stromal cell derived factor-1
    b. Functions to release vWf from the intracellular space by actions on the endothelial vasopressin receptor
    c. Functions to release microparticles from platelets carrying active vWf by signaling though platelet surface vasopressin receptors
    d. Functions to release an excess of thrombopoietin from hepatocytes by vasopressin receptors on zone 1 hepatocytes

2. vWf acts to bridge the endothelium to the platelet by interaction with
    a. Platelet glycoprotein IIb subunit of the IIbIIIa receptor
    b. Platelet glycoprotein IIIa subunit of the IIbIIIa receptor
    c. Platelet glycoprotein 1b subunit of the 1b/V/IX receptor
    d. Platelet glycoprotein IX subunit of the 1b/V/IX receptor

3. Functional analysis along with quantitative analysis of the vWf antigen is recommended for
    a. Patients suspected to have type 2 vWf deficiency
    b. Patients with no clinical bleeding but deficient vWf determined on quantitative analysis
    c. Patients with no vWf on quantitative analysis
    d. Patients with thrombocytosis and normal vWf activity

4. Name four methods of platelet enumeration

5. Essential Thrombocythemia is typically characterized with
    a. Thrombocytopenia
    b. Marrow megakaryocyte hyperplasia
    c. Erythrocytosis
    d. Leukocytosis