TOPIC: Lec 09 Platelet Disorder

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Platelet Disorder

• The hemostatic system consists of **platelets**, **coagulation factors**, and the **endothelial cells** lining the blood vessels.

• The platelets arise from the fragmentation of the cytoplasm of **megakaryocytes** in the bone marrow and circulate in blood as disc-shaped anucleate particles for **7-10 days**.
Platelet Disorder

• Bleeding from platelet disorders may be due to either:
  • **Quantitative abnormalities** i.e. thrombocytopenia
  • **Qualitative abnormalities** i.e. defective platelet function

• Thrombocytopenia is an **abnormality** of platelets

• It may be **congenital** or **acquired** defect

• It may cause a **thrombotic** or a **bleeding** tendency or may be part of a wider disorder such as **myelodysplasia**
• Bleeding from platelet disorders typically characterized by bleeding from:
  • **mucous membranes** e.g. gum bleeds, epistaxis, menorrhagia
  • **skin** - petechiae, purpura, ecchymoses
  • **Petechiae**: pinpoint red lesions less than 2mm in size
  • **Purpura**: lesions 2mm - 1cm in size
  • **Ecchymoses**: lesions greater than 1cm in size
  • prolonged bleeding following trauma and surgery.
Causes of Platelet Dysfunction

**Congenital**
- Bernard Soulier
- Glanzman’s
- Storage pool disease
- Gray Platelet syndrome
- Von Willenbrand Disease
- Platelet release disorders

**Acquired**
- Uremia
- Medication
  - NSAIDS (Acetylsalicylic acid, ibuprofen, etc)
  - Clopidogrol
- Trauma
- Cardiac Surgery
- Liver failure
- Myeloproliferative disorders
- Myelodysplasia
Platelet structure

**Plasma (surface) membrane protein:**

- GPIIb-IIIa – fibrinogen receptor
- GP Ib-IX-V: vWF receptor
- GPIa-IIa: collagen receptor
Bernard-Soulier Syndrome

• Is a deficiency of glycoprotein protein Ib, which mediates the initial interaction of platelets with the subendothelial components via the von Willebrand protein.
• It is a rare but severe bleeding disorder.
• The platelet count is low, but, characteristically, the platelets are large.
PATHOPHYSIOLOGY

• The platelet membrane contains specific glycoprotein (GP) receptors, which function in platelet adhesion, activation, and aggregation.

• The GPIb-IX-V receptor complex, is responsible for platelet adhesion through its interaction with von Willebrand factor on the exposed subendothelium.

• The molecular defect involves the absence of a platelet membrane glycoprotein (platelet membrane von Willebrand factor) leading to defective platelet adhesion.

• This has been found to be caused by mutation in the GP1BA gene, the GP1BB gene, or the GP9 gene.

• It is familial with autosomal recessive inheritance.
CLINICAL MANIFESTATIONS

• Bernard-Soulier syndrome presents early with bleeding symptoms, most commonly
  – epistaxis
  – ecchymosis
  – cutaneous and gingival bleeding
  – menometrorrhagia
  – gastrointestinal bleeding.

• Rarely, patients will have severe hemorrhage at times of injury or surgery.

• The severity of these bleeding symptoms is variable among patients and may range from mild to life-threatening.

• Heterozygous patients may have mild to moderate bleeding tendencies.
Epidemiology

• The syndrome is rare - estimated prevalence is less than 1 per million. Consanguineous marriages have been reported in the families of 81% of patients.
Investigations

• FBC and film: platelet count is usually low but may be normal. Giant platelets are seen on the blood film.

• Bleeding time is prolonged and may be longer than 20 minutes.

• Platelet aggregation studies: platelets do not aggregate in response to ristocetin or von Willebrand factor.
• Peripheral blood smear will reveal large platelets
  – Typically more than one third of the platelets are about half of the size of a red blood cell (3.5 micrometre)
  – Some platelets are as large or larger than a lymphocyte

• Bone marrow biopsy - normal numbers of megakaryocytes without significant morphologic abnormalities
• Modern platelet function tests, such as the PFA-100, may be useful but with variable sensitivity, depending on the severity of the defect.
• Flow cytometry can demonstrate abnormalities of platelet membrane glycoprotein.
• Flow cytometry can be used to confirm defects in the GPIb-IX-V complex by antibodies directed against platelet surface antigen CD42b, revealing a severe reduction or deficiency of GPIb.
Treatment

• In general, no medications are needed. Treatment of bleeding episodes includes:
• Antifibrinolytic agents, eg epsilon-aminocaproic acid, may be used for mucosal bleeding.
• For surgery or life-threatening haemorrhage, platelet transfusion is the only available therapy for surgery or life-threatening bleeding.
• Desmopressin acetate (DDAVP®) has been shown to shorten the bleeding time in some patients with Bernard-Soulier syndrome. It does not work for all patients.
• Recombinant activated factor VII has also been used.
Glanzman’s

- Is inherited in an autosomal recessive manner.
- The genes of both of these proteins are on chromosome 17.
- Different genetic mutations of either GP IIb or IIIa genes result in a heterogeneity of thrombasthenia phenotype.
- Carrier detection in GT is important to control the disease in family members.
- Can be acquired as an autoimmune disorder
Pathogenesis

• Platelet glycoprotein IIb/IIIa (GP IIb/IIIa) complex is deficient or present but dysfunctional.
• Defect in the GP IIb/IIIa complex leads to defective platelet aggregation and subsequent bleeding.
• Aggregation of PLTs occurs in response to ristocetin, but not to other agonists such as ADP, thrombin, collagen or epinephrine.
Symptoms

• purpura
• Menorrhagia
• Mucosal bleeding
• xBrain hemorrhages
• epistaxis
• gingival bleeding
• gastrointestinal bleeding
• postpartum bleeding
• increased bleeding post-operatively
DIAGNOSIS

• Normal PLT count and morphology.
• Greatly prolonged bleeding time.
• Absence of PLT aggregation in response to ADP, collagen, epinephrine or thrombin (Platelet aggregation test)
• Flow cytometry (CD 41, CD 61).
• Studies of GP IIb/IIIa receptors on the PLT membrane.
Treatment

• Dental hygiene lessens gingival bleeding
• Avoidance of antiplatelet agents such as aspirin and other antiinflammatory drugs (NSAIDs) such as ibuprofen and naproxen, and anticoagulants
• Iron or folate supplementation
• Antifibrinolytic drugs such as tranexamic acid or ε-aminocaproic acid
• Desmopressin (DDAVP) does not normalize the bleeding time in Glanzmann's thrombasthenia but anecdotally improves hemostasis
• Hormonal contraceptives to control excessive menstrual bleeding - Platelet transfusions (only if bleeding is severe; risk of platelet alloimmunization)
• Recombinant factor VIIa
• Hematopoietic stem cell transplantation (HSCT) for severe recurrent hemorrhages
Platelet Storage Pool Disease

- Classified by type of granular deficiency or secretion defect acetylsalicylic acid.
- Dense body deficiency, alpha granule deficiency (gray platelet syndrome), mixed deficiency, Factor V Quebec
- Secretory granules:

A-Granules

Dense granules

Lysosomes
pathophysiology

- pathophysiology of platelet storage pool deficiency one must consider several factors including the human body's normal function prior to such a deficiency, such as platelet alpha-granules one of three types of platelet secretory granule
- Platelet α–granules are important in platelet activity, α–granules connect with plasma membrane. This in turn increases the size of the platelet. Platelet α–granules have an important role in hemostasis as well as thrombosis.
- insulin-like growth factor 1, platelet-derived growth factors, TGFβ, platelet factor 4 (which is a heparin-binding chemokine) and other clotting proteins (such as thrombospondin, fibronectin, factor V,[2] and von Willebrand factor).[3]
Diagnosis

• The diagnosis of this condition can be done via the following:

  • Flow cytometry
  • Bleeding time analysis
Types

- This condition may involve the alpha granules or the dense granules. Therefore the following examples include:

- Flow cytometry analysis
- Platelet alpha-granules
  - Gray platelet syndrome
  - Quebec platelet disorder
- Dense granules
  - δ-Storage pool deficiency
  - Hermansky–Pudlak syndrome
  - Chédiak–Higashi syndrome
Treatment

• Platelet storage pool deficiency has no treatment however management consists of antifibrinolytic medications if the individual has unusual bleeding event, additionally caution should be taken with usage of NSAIDS.
Storage Pool Disorders
Gray Platelet Syndrome

- Absence of \( \alpha \)-granules, Molecular defect unknown, Mild mucocutaneous bleeding, Variably prolonged bleeding time, Moderate thrombocytopenia, Reticulin fibrosis of Bone Marrow, Large gray platelet
Quebec Platelet Disorder

- QPD is a rare, autosomal dominant bleeding disorder described in a family from the province of Quebec in Canada.
- Large amounts of the fibrinolytic enzyme urokinase-type plasminogen activator (u-PA) in platelets.
- Stored platelet plasminogen is converted to plasmin.
Storage Pool Disorders
Dense Granule Disorders

• **Normal** dense granules, 3-6/ platelet, Serotonin, ADP, ATP, Ca,
  **Heterogeneous** group of disorders, Molecular defect **unknown**, Mild to moderate **bleeding**
Storage Pool Disorders

• Two autosomal recessive syndromes associated with albinism
  Chediak-Higash
  Hermansky-Pudlack
• Non-albino syndromes
  Wiskott-Aldrich
  Thrombocytopenia absent radii
  Osteogenesis imperfecta
Storage Pool Disorders

Chediak-Higashi
• Partial oculocutaneous albinism
• Frequent pyogenic infection
• Giant lysosomal granules in cells
• Thrombocytopenia
• Dense granule deficiency

Hermansky-Pudlak
• Oculocutaneous albinism
• Inclusions in the cells of reticuloendothelial system
• Thrombocytopenia
• Dense granule deficiency
Storage Pool Disorders

- Clinical presentation
- Platelet morphology normal
- Bleeding time usually, not always prolonged
- Aggregation
  - Marked impairment with weak agonists ADP, epinephrine and low concentrations of collagen
  - Response to higher concentration may be normal
  - Absent second wave of aggregation when stimulated by ADP and epinephrine
Von Willebrand Factor

• The gene for vWF → chromosome 12p.
• Synthesized in endothelial cells and megakaryocytes and stored in Weibel-Palade bodies and platelet alpha granules, respectively.
• vWF is initially formed in the ER as a pre-pro VWF molecule, which would assemble into homomultimeric protein after glycosylation, dimerization and multimerization in the golgi organelle and storage places in the cells.
**Function of vWF**

- Serves as the carrier protein for factor VIII (probably factor VIII and vWF are brought together in storage granules).
- Serves as the ligand that binds to glycoprotein Ib receptor on platelets to initiate platelet adhesion to damaged blood vessel walls.
- vWF needs to be activated to be able to bind to GP Ib receptor on platelets (Ristocetin, high shear force, collagen, etc)
von Willebrand disease:

- **Pathogenesis:** defect of platelet GPIba results in an increased desire for normal vWF leading to the binding of the largest vWF multimers to resting platelets and to their clearance from the circulation, resulting in thrombocytopenia and adhesion defect.

- **Inheritance:** autosomal dominant

- **Laboratory findings:**
  - Prolonged bleeding time
  - Moderate thrombocytopenia
  - Loss of large vWF multimers
  - Enhanced ristocetin-induced platelet aggregation

- **Differential diagnosis:** from Type 2B vWD (molecular characterisation of platelet GPIba)

- **Treatment:** platelet concentrate + vWF concentrate.
VON WILLEBRAND DISEASE
I/2. Disease associated platelet function disorders

Uremia: complex *hemostatic defect*
– thrombocytopenia, platelet dysfunction (adhesion, aggregation, secretion defects), mild coagulation abnormalities.

• Hematopoetic disorders:
  – paraproteinemias, myeloproliferative disorders, myelodysplastic syndrome, leukemia.

• Cardiopulmonary bypass operation

• Platelet antibodies:
  – auto-, alloantibodies

• Others:
  – diabetes mellitus, liver disease, DIC
Collagen receptor deficiency

- **Pathogenesis:**
- abnormalities of platelet *GPVI* and *GPIa-IIa* (receptors for collagen)
- defect of adhesion and collagen-induced platelet aggregation.
Platelet ultrastructure

- Electron dense granule containing nucleotides (ADP), Ca^{2+}, serotonin
- Submembranous filaments (platelet contractile protein)
- Specific alpha granule containing growth factor, factor V, VWF, fibronectin, beta-thromboplastic, heparin antagonist, thrombospondin
- Plasma membrane
- Open canalicular system
- Platelet phospholipid
- Glycocalyx
- Dense tubular system
- Glycogen
- Mitochondria
B: Defects of intracellular signal transduction and secretion

- Abnormalities of the arachinodate/thromboxane A2 pathway® platelet function defects, mild bleeding.
- Impaired liberation of Arachidonic acid from membrane phospholipids
- Cyclooxygenase deficiency ("aspirin like disease")
- Thromboxane synthetase deficiency
- Thromboxane A2 receptor abnormalities
Disorders of receptors and signal transduction:

- Cyclooxygenase inhibitors (TXA2 – e.g. Aspirin)
- Adenosine diphosphate inhibitors (ADP)
- GPIIb-IIIa receptor antagonists
Therapy

- Platelet **transfusion** should be used only in **severe bleeding** episodes
- Recombinant factor VIIa
- **Antifibrynolytic agents** (tranexamic acid)
- Desmopressin (DDAVP)
Thrombocytopenia

Abnormal bleeding due to thrombocytopenia or abnormal platelets function is also characterized by spontaneous skin purpura & hemorrhage & prolonged bleeding after trauma.

A. Decreased marrow **production** of megakaryocytes
   - congenital disorders
   - acquired disorders

B. **Splenic sequestration** of circulating platelets

C. Increased **destruction** of circulating platelets
   - (congenital/acquired disorders)
   - **immune** destruction
   - **nonimmune** destruction
Thrombocytopenia (A)

A. **Decreased** marrow production of **megakaryocytes**

- **congenital** disorders
  - Fanconi’s anemia ⇒ genetic defect in a cluster of proteins responsible for DNA repair
  - thrombocytopenia with absent radii (TAR) ⇒ absence of the radius bone in the forearm, and a dramatically reduced platelet count

- **acquired** disorders
  - marrow **infiltration** with malignant cells
  - marrow **fibrosis**
  - **aplastic** and **hypoplastic** anemias (idiopathic, drugs, toxins)
  - **deficiency** states (vitamin B12, folate, iron)
Thrombocytopenia (B)

B. Splenic **sequestration** of circulating platelets

- splenic enlargement due to **tumor infiltration**
- splenic enlargement due to **portal hypertension**
Thrombocytopenia (C)

C. Increased destruction of circulating platelets

- **congenital** disorder
  - **Wiscott-Aldrich** syndrome → rare **X-linked** recessive disease → eczema, thrombocytopenia, immune deficiency, and bloody diarrhea (secondary to the thrombocytopenia).
  - **Bernard-Soulier** syndrome
Thrombocytopenia (C)

• **acquired** disorders
  - **nonimmune destruction**
    - Disseminated intravascular coagulation (DIC) → pathological activation of coagulation
    - hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura
    - Sepsis
    - vascular prostheses, cardiac valves
  - **immune destruction**
    - Primary immune thrombocytopenic (ITP)
    - drug-induced thrombocytopenia
    - chronic autoimmune disorders
    - infection (HIV)
    - malignancies
Thrombocytosis

• Thrombocytosis resulting from myeloproliferation
  – essential thrombocythemia
  – polycythemia vera
  – chronic myelogenous leukemia
  – myeloid metaplasia

• Secondary (reactive) thrombocytosis
  – systemic inflammation
  – malignancy
  – iron deficiency
  – hemorrhage
  – postsplenectomy
HEPARIN-INDUCED THROMBOCYTOPENIA (HIT)

- caused by antibodies directed against heparin in complex with platelet factor 4
- 50% or more reduction in platelet count
- Beginning 5 or more days after first exposure to heparin
- Thrombotic complications
- Therapy – to discontinue all forms of heparin
- Direct IIa inhibitors (lepirudin, argatroban) and Xa (danaparoid)