Platelet disorders

Dr. Klara Vezendi
Szeged University
Transfusiology Department
Hemorrhagic platelet disorders are caused by:

- either quantitative deficiency of circulated platelets (thrombocytopenia, thrombocytosis)

- a qualitative defect in platelet function or

- a combination of both.

Symptoms:

- spontaneous cutaneous and mucosal bleeding and purpura

- prolonged bleeding following trauma and surgery.
A. Quantitative platelet disorders
Normal platelet count: (100) 150 - 350 x 10⁹/l.

**Thrombocytopenia** is defined as a platelet count is less than (100) 150 x 10⁹/l.

**Thrombocytosis**: platelet count is > 350 x 10⁹/l.

*With normal platelet function, thrombocytopenia is rarely the cause of bleeding unless the count is less than 50 x 10⁹/l.*
Causes of thrombocytopenia I.

• **Insufficient platelet production**
  
  – Multilineage bone marrow failure (leukemia, lymphoma, myelofibrosis, aplastic anemia, carcinoma, cytomegalovirus infection)
  
  – Selective megakaryocyte (MK) disruption (viral infections, gold therapy, alcohol intoxication, amegakaryocytic thr.-penia)
  
  – Hereditary thrombocytopenia (Bernard-Soulier, Wiskott-Aldrich sy.)

• **Platelet sequestration** (hypersplenism due to portal hypertension or infiltrative disease)
Causes of thrombocytopenia II.

- **Increased peripheral platelet destruction**
  - Autoimmune: ITP, SLE
  - Alloimmune: post-transfusion purpura, neonatal
  - Drug-induced: heparin-induced thrombocytopenia, antibiotics
  - Over consumption: DIC, TTP, HUS, extracorporal circulation

- **Other**
  - Dilution (massive transfusion)
  - Artifactual (in vitro platelet clumping)
Immune thrombocytopenic purpura (ITP)

Pathophysiology: formation of auto- antibodies against specific membrane glycoproteins (GPIIa, IIb or Ib).

The autoantibodies coated platelets are phagocytized by RES.

Increased destruction of platelets combined with inadequate compensation by the bone marrow results in thrombocytopenia.
Clinical forms:
- acute ITP: often follows a viral illness, mostly in children, mild and self-limited and uncommonly requires specific therapy.
- chronic ITP: more commonly in adults, moderate to severe thrombocytopenia with clinical findings that require medical treatment.

Diagnosis: requires the exclusion of other diseases associated with thrombocytopenia (e. g. leukemia, thrombotic thrombocytopenic purpura /TTP/, drug reactions and myelodysplastic syndrome).
Laboratory: thrombocytopenia

large, immature platelets are often in peripheral blood smear

Bone marrow: MK-s are present

Platelet- associated IgG antibodies in the serum: test sensitivity 49-66% → negative result does not rule out the diagnosis.

Clinical features:

platelet-type (mucosal) bleeding, „wet purpura” and the absence of splenomegaly supports the diagnosis.

Bleeding is often less pronounced than in cases of decreased production with similar platelet counts.
Peripheral blood smear of a patient with ITP: decreased number of platelets and a large platelet in the middle.

Bone marrow: MK-s are present.
ITP

„wet purpura”
Treatment: is necessary:

in the asymptomatic patient with platelet count <30 x10⁹/l, in the symptomatic patient: 30-50 x10⁹/l.

- **Corticosteroid**: prednisone (D: 1-2 mg/kg/day), response rate: 50-60%
- **Splenectomy**: if steroid is ineffective after 3-6 months. Laparoscopic mode, 2-3 weeks before operation: Pneumococcal vaccination. Response rate: 66%
- **High dose iv. gammaglobuline (IVIG)**: 1 g/kg/day for 2 to 3 days
- **Rh(D) immune globulin** for Rh-positive patients (75 μg/kg)
- Relapsed ITP (platelet count <30 x10⁹/l):
  Rituximab, a monoclonal antibody to CD20. New drugs: **TPO receptor agonists** (Romiplostin).
**Post-transfusion purpura:** alloimmune

7-10 days after platelet transfusion severe thrombocytopenia.

*Pathophysiology:*

*Platelet-specific antibodies in the recipient’s serum* – most frequently against HPA-1a antigen.

Patients become sensitized to platelet antigen from prior transfusion of platelet-containing products or from pregnancy. This explains the much higher incidence among women.

*Treatment:* **IVIG** (400 mg/kg/day for 5 days or 1 g/kg/day for 2 days), **plasmapheresis**

Additional platelet transfusions can worsen the thrombocytopenia! Further transfusions should be washed or HPA-1a antigen–negative.
Neonatal alloimmune thrombocytopenia (NAIT): a potentially severe disease caused by fetomaternal incompatibility for platelet specific antigens.

Pathogenesis: it is due to the presence of fetal platelet-specific antigens (HPA-1a) that are not expressed on maternal platelets.

As a result of fetomaternal bleeds, fetal platelets entry into the maternal circulation → alloantibodies are produced by the mother.

These antibodies cross the placenta and destroy fetal platelets → thrombocytopenia, risk of intracranial hemorrhage.

Treatment: in utero transfusion of HPA-1a-negative platelets.

Fetal scalp vein platelet count estimations at the time of delivery to determine the mode of delivery (cesarean section?).
Heparin-induced thrombocytopenia (HIT):

Type I: within the first 2 days after exposure to heparin, is a non-immune disorder, and it occurs with the direct effect of heparin (platelet agglutination). No clinical consequences. About 10 % of patients receiving heparin.

Type II: 4-10 days after heparin treatment, is an immune-mediated disorder.

Pathophysiology: antibody formation against the heparin-platelet factor 4 complex. The antibodies bind to the surface of platelets and induce their activation and activate endothelial cells → thrombosis (is usually venous: DVT, PE, rarely arterial: stroke, MI)

No bleeding. The mortality rate is approximately 20%.

Incidence: 0,3-3%, most commonly with unfractionated heparin, has no relation to the heparin dose.
Diagnosis of HIT Type II:
 mostly clinical.

Laboratory: serotonin release assay (expensive, high sensitivity and specificity),
heparin-induced platelet aggregation test (HIPA) low sensitivity, high specificity)

Treatment:
- stop heparin
- use alternative anticoagulants:
  direct thrombin inhibitors (argatroban, hirudin)
  anti Xa drugs (danaparoid)
  oral anticoagulants
Drug induced purpuras (Vancomycin)
Thrombotic microangiopathies: TTP, HUS

Acute, fulminant disorders characterized by thrombocytopenia and microangiopathic hemolytic anemia.

Pathophysiology is the same, clinical picture is different.
Thrombotic thrombocytopenic purpura (TTP, Moschcowitz syndrome) I.

- A pentad of signs:
  - thrombocytopenia
  - microangiopathic hemolytic anemia
  - fever
  - renal dysfunction
  - neurologic signs.

- A clinical triad:
  - thrombocytopenia
  - red cell fragments (schistocytes)
  - increased lactate dehydrogenase (LDH)

![Schistocyte Image]
Thrombotic thrombocytopenic purpura (TTP, Moschcowitz syndrome) II.

**Causes:**

- **Idiopathic:** is recently linked to the inhibition of the enzyme ADAMTS13 by antibodies (*autoimmune disease*). ADAMTS13 is a metalloprotease responsible for the breakdown of vWF. Very large vWF molecules are more prone to lead to coagulation.

- **Secondary:** 40% of all cases of TTP

Cancer, bone marrow transplantation, pregnancy, HIV-infection, drugs (quinine, platelet aggregation inhibitors: ticlopidine, clopidogrel, immunosuppressants).

**Treatment:** **plasma exchange** (40-60 ml/kg/day) **immunosuppressive drugs** (glucocorticoids, vincristine, cyclophosphamide)

Refracter or relapsed case: rituximab, splenectomy.

No platelet transfusion!
Hemolytic uremic syndrome (HUS, Gasser disease)

It is a disease with hemolytic anemia, acute renal failure (uremia) and thrombocytopenia. It predominantly but not exclusively affects children.

**Causes:**
- classical form: 90 % of cases
  D-HUS (post-diarrhoeal HUS) occurs after bloody diarrhoea caused by a strain of E. coli that expresses verotoxin (Shiga-like toxin).

Toxin enters bloodstream, attaches to glomerular endothelium → acute renal failure.
Damage to blood vessels in all tissues → microangiopathies.
Activate platelets → thrombocytopenia.

- less common form: 10 % of cases (adult HUS, familial HUS)
No diarrhoea.
B. Qualitative platelet disorders
Qualitative *(functional)* platelet disorders are suggested by a **prolonged bleeding time** (abnormal platelet function screen) or clinical evidence of **bleeding** in the setting of a **normal platelet count** and coagulation studies.

Platelet dysfunction is frequently associated with excessive bleeding.
**Platelet morphology** may be characteristic of certain types of the thrombocytopathy

**Normal platelets**
(peripheral blood smear)
Giant platelets:
size > RBC
Found in:
increased platelet turnover
myeloproliferative sy.
MDS

Large platelets:
size < RBC, but > 1/3 RBC
Found in:
increased plt. turnover
myeloproliferative sy.
MDS
Bernard-Soulier sy.
May Hegglin anomaly
Gray platelet sy.
Degranulated, gray coloured platelets:

Found in:

- gray platelet sy.
- discharge of platelet granules
  - *in vivo* (cardiopulmonary bypass, hairy cell leukemia)
  - *in vitro* (poor venesection technique)

Small platelets:

Found in:

Wiskott Aldrich sy.
Functional platelet disorders

• **I. Acquired:** common
  – 1. Drug induced platelet dysfunction
  – 2. Disease associated platelet dysfunction

• **II. Inherited:** rare (frequency is probably underestimated because diagnostic difficulties)
  – 1. Adhesion defects:
    • Bernard – Soulier sy.
    • Platelet type vW
    • Collagen receptor deficiency
  – 2. Aggregation defects:
    • Glanzmann thrombasthenia
    • Afibrinogenemia
  – 3. Secretion disorders:
    • Granule abnormalities (α, δ, α+δ)
    • Defects of signal transduction
  – 4. Defects of platelet coagulant activity:
    • Scott sy.
I. Acquired disorders of platelet function
I/1. Drug induced platelet dysfunction

- **Analgesics**
  - Aspirin (irreversible inhibition of cyclooxygenase /COX/ enzym), 15-30 min after ingestion 40-100 mg, persists 4-5 days
  - Nonsteroidal anti-inflammatory drugs /NSAIDs/ (reversible COX inhibition)

- **Ticlopidin (Ticlid), clopidogrel (Plavix)**
  - Block platelet- ADP receptors, 24-48 hours after ingestion, persists 10 days

- **GPIIb/IIIa receptor antagonists** (→ acquired Glanzmann)

- **Dipyridamol**
  - Elevates cAMP level → PGI2 (prostacyclin) effect↑: inhibits platelet aggregation

- **β-lactam antibiotics**
  - penicillin, cephalosporin

- **Cardiovascular drugs**
  - Ca antagonists, nitroglycerin, propranolol, isosorbid dinitrate

- **Psychotropic drugs**
  - antidepressants, phenothiazins

- **Others:** plasma expanders, antihistamins, cytostatic agents, heparin, fibrinolytics
Mechanism of antiplatelet agents

- Clopidogrel
- Dipyridamole
- ADP
- Prostacyclin
- Collagen
- Thrombin
- Thromboxane A₂
- GP IIb/IIIa
- Aspirin
- IIb/IIIa antagonists
- COX
- Activation
- Thromboxane A₂

- Pathway:
  - ADP triggers activation, leading to Thromboxane A₂ production.
  - Clopidogrel blocks ADP receptor.
  - Dipyridamole increases Prostacyclin production, which inhibits Thromboxane A₂.
  - Aspirin inhibits COX, reducing Thromboxane A₂ production.
  - GP IIb/IIIa antagonists prevent platelet aggregation.

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
Functional platelet disorders

I. Acquired: common
   - 1. Drug induced platelet dysfunction
   - 2. Disease associated platelet dysfunction

II. Inherited: rare (frequency is probably underestimated because diagnostic difficulties)
   - 1. Adhesion defects:
     - Bernard–Soulier sy.
     - Platelet type vW
     - Collagen receptor deficiency
   - 2. Aggregation defects:
     - Glanzmann thrombasthenia
     - Afibrinogenemia
   - 3. Secretion disorders:
     - Granule abnormalities ($\alpha$, $\delta$, $\alpha+\delta$)
     - Defects of signal transduction
   - 4. Defects of platelet coagulant activity:
     - Scott sy.
II. Inherited platelet disorders
II/1. Adhesion defects

- Bernard – Soulier syndrome
- „Platelet type” (pseudo) vWD
- Collagen receptor deficiency
**Bernard – Soulier syndrome:**

*First case:* 1948 (J. Bernard, JP Soulier)

*Pathogenesis:* absence or decreased expression of the glycoprotein complex $\text{GP Ib-IX-V}$ on the surface of the platelets. 

(Ib$\alpha$, Ib$\beta$, GPIX, GPV)

A GPIb$\alpha$ is the receptor of von Willebrand factor $\Rightarrow$ deficient binding of vWF to the platelet membrane at sites of vascular injury, resulting in defective platelet adhesion.
**Inheritance:** autosomal recessive

**Incidence:** <1: 1 million

**Clinical presentation:** epistaxis, ecchymoses, menorrhagia, gingival bleeding, gastrointestinal bleeding.

**Laboratory findings:**
- Prolonged bleeding time
- moderate thrombocytopenia (platelet life time↓)
- platelet aggregation by ristocetin is deficient (ADP, arachidonic acid, epinephrin, collagen: norm.)
- clot retraction: norm.
- abnormally large platelets (>3,5 μm - 20-30 μm)
„Platelet type” (pseudo) von Willebrand disease:

Pathogenesis: defect of platelet GPIbα ⇒ increased avidity for normal vWF ⇒ leading to the binding of the largest vWF multimers to resting platelets and to their clearance from the circulation ⇒ results 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 dg: from Type 2B vWD (molecular characterisation of platelet GPIbα)

Treatment: platelet concentrate + vWF concentrate.
Collagen receptor deficiency

Pathogenesis:

abnormalities of platelet *GPVI and GPIa-IIa* (receptors for collagen)

⇒ defect of adhesion and collagen-induced platelet aggregation.
II/2. Platelet aggregation defects

- Glanzmann’s thrombasthenia
- Afibrinogenemia
Glanzmann’s thrombasthenia:

Pathogenesis: abnormalities of platelet **GPIIb-IIIa** (fibrinogen receptor) $\Rightarrow$ platelets less able to adhere to each other and to the underlying tissue of damaged blood vessels.
First case: 1918 (Glanzmann)

Inheritance: autosomal recessive manner

Types: Type 1: absence of GPIIb-IIIa (< 5%)
Type 2: reduced surface expression of GPIIb-IIIa (10-20%)

/Essentialis athrombia: clot retraction is normal/

Type 3: dysfunction of GPIIb-IIIa

Laboratory features: - normal platelet count and morphology
- platelet aggregation occurs in response to ristocetin, but not to other agonists (such as ADP, thrombin, collagen or epinephrine).
- clot retraction in absent

Symptoms: mucocutaneous bleeding in the neonatal period, menorrhagia, ecchymoses, epistaxis, gingival hemorrhage.
Afibrinogenemia:

Inheritance: autosomal recessive, incidence: 1:2 million

Pathogenesis: fibrinogen helps platelets to glue together to form the initial “plug” in response to an injury → in afibrinogenemia: combined bleeding disorder because both platelets and clotting are abnormal.

Laboratory findings:
- absence of fibrinogen from the blood
- abnormal platelet aggregation, bleeding time, APTT, PT, TT
- may be moderate thrombocytopenia

Clinical symptoms: bleeding umbilical cord, bruising, nosebleeds, gastrointestinal bleeding, miscarriage, excessive bleeding after injury or surgery.
II/3. Abnormalities of platelet secretion

- A. Abnormalities of platelet granules
  - α: gray platelet sy, FV Quebec, Jacobsen- Paris-Trousseau sy.
  - δ: storage pool deficiency, Hermansky-Pudlak sy, Chediak-Higashi, Wiskott-Aldrich, TAR
    - α and δ combined deficiency

- B. Abnormalities of the signal transduction and secretion
  - abnormalities of the arachidonate/thromboxane A2 pathway
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-thromboplastin, heparin antagonist, thrombospondin
- Glycocalyx
- Dense tubular system
- Glycogen
- Mitochondria
- Plasma membrane
- Open canalicular system
- Platelet phospholipid
A: Abnormalities of platelet granules I.

**Abnormalities of platelet α-granules:**

- **Gray platelet syndrome:** very rare disorder
  
  **Pathogenesis:** marked *decrease or absence of platelet α-granules and of platelet-specific α-granule proteins* ⇒ no PF4, βTG, PDGF secretion

  **Symptoms:** lifelong mild or moderate mucocutaneous bleeding tendency.

  **Platelet morphology:** large and contain few granules, giving them a *gray appearance* in light microscopy with the May-Grünwald-Giesma stained blood film.
Quebec platelet disorder (FV Quebec):

Pathogenesis: abnormal proteolysis of α-granule proteins (FV, fibrinogen, thrombospondin, multimerin, vWF, P-selectine), probably is due to u-PA activity.

First case: 1974 (Tracy)

Inheritance: autosomal dominant

Laboratory findings: moderate thr. penia, normal plazma FV activity, decreased platelet-FV activity, normal platelet FV antigen. Urokinase-plasminogen activator (u-PA) expression ↑.

Normal morphology of α-granules.

Symptoms: severe post-traumatic bleeding manifestations, unresponsive to platelet transfusion.

Therapy: antifibrinolytic agent.
• Jacobsen -Paris-Trousseau syndrome:

Pathogenesis: platelets have giant $\alpha$-granules, which are unable to release their content upon platelet stimulation.

Deletion of the distal part of chromosome 11.

First case: 1973 (Jacobsen)

Rare syndrome, ~100 cases.

Laboratory findings: moderate thr. penia,
  giant $\alpha$-granules in circulating platelets

Clinical symptoms: mucosal bleeding, mental retardation, may be multiple congenital anomalies (cardial, genitourinal).
A: Abnormalities of platelet granules II.

**Abnormalities of platelet Delta (δ, dense) - granules:**

- **Storage pool disease:**
  
  *isolated deficiency of δ- granules* in megakaryocytes and platelets. Inheritance: autosomal recessive or dominant. Mild to moderate bleeding tendency.

- **Hermansky-Pudlak syndrome:**
  
  Inheritance: autosomal recessive Bleeding diathesis.

  δ-granules deficiency + others: oculocutaneous albinism (decreased pigmentation – skin, hair), eye problems (strabism, photophobia, nystagmus, impaired vision), inflammatory bowel disease, progressive pulmonary fibrosis (major complication, fetal).
Hermansky Pudlak syndrome
(oculocutaneous albinism)
● **Chediak-Higashi syndrome:**

- Decrease in platelet δ-granules,
- partial oculocutaneous albinism,
- dysfunctional neutrophils with giant lysosomal granules,
- abnormal natural killer cell function, impaired bacteriolysis
  ⇒ recurrent **pyogenic infections.**
- Mild bleeding diathesis.
**Wiskott-Aldrich syndrome:**

Rare (4-10:1 million), X–linked recessive disease → the overwhelming majority are male.

*Pathogenesis:* mutations in the WASP family (is expressed exclusively in hematopoietic cells).

*Clinical symptoms:* microthrombocytopenia, bleedings, immunodeficiency, extensive eczema, recurrent infections (due to impaired function of lymphocytes and neutrophils), malignancy (lymphoma, leukemia).

Milder form of the disease: „X-linked thr. penia” (without immunodeficiency and eczema).
Wiskott Aldrich syndrome

Small platelets in blood film.
Prognosis: poor.
The average individual lives about 6,5 years; those who survive into adolescence often develop cancer. Death usually occurs from severe bleeding or overwhelming infection in the first few years of life.

Treatment: - transfusions of platelets
- antibiotics
- iv. infusions of immune globulin
- splenectomy (severe thrombocytopenia with bleeding)
- bone marrow transplantation with HLA-identical marrow

Gene therapy is promising.
Thrombocytopenia absent radius (TAR) syndrome:

Pathogenesis: a congenital malformation syndrome characterised by bilateral absence of the radii and a thrombocytopenia.

Inheritance: autosomal recessive.

Incidence: <1:100000
Other findings: hypomegakaryocytic neonatal thrombocytopenia (due to defective, thrombopoiesis), δ granules deficiency, other abnormalities: gastrointestinal, skeletal, hematologic, cardiac system.

Lower limb involvement in two children with TAR syndrome.

Symptoms: easy bruising, hemorrhage (the major cause of mortality). The incidence of hemorrhage is limited to the first 14 months of life.
B: Defects of intracellular signal transduction and secretion

Abnormalities of the arachidonic acid/thromboxane A$_2$ pathway → platelet function defects, mild bleeding.

- Impaired liberation of Arachidonic acid from membrane phospholipids
- Cyclooxygenase deficiency („aspirin like disease”)
- Thromboxane synthetase deficiency
- Thromboxane A$_2$ receptor abnormalities
II/4. Disorders of platelet procoagulant activity

- **Scott syndrome**: rare bleeding disorder.
  - *Inheritance*: autosomal recessive

**Pathogenesis**: is linked to the *lack of exposure of procoagulant phosphatidylserine (PS) to the external leaflet of the plasma membrane* of activated platelets and other hematologic lineages → impaired thrombin formation.

**Symptoms**: defective wound healing, bleeding tendency.
Treatment of qualitative platelet disorders I.

• **Desmopressin (DDAVP):** is a vasopressin analog, improves hemostasis without the risks of transfusion. It releases FVIII and large multimeres of vWF from tissue stores.
  - D: 0.3-0.4 μg/kg (in 50 ml NaCl infusion, during 15-30 min).
  - Ineffective in patients with Scott syndrome and Glanzmann’s thrombasthenia.
  - No in Pseudo (platelet type) vWD.

• **Transfusion of normal platelets:**
  - D: 1E/10 kg (pooled), 0.5 x10^11 thr/10 kg (apheresis)
  - It should be reserved for life-threatening bleeding because of the development of alloantibodies.
  - Because bleeding is a lifelong problem, HLA-matched platelets should be considered (apheresis).
  - Risk of transfusion - transmitted infections (viral, bacterial).

• **rFVIIa:** in severe bleeding (D: 90 μg/kg)
Treatment of qualitative platelet disorders II.

- **Adjuvant treatment:**
  - Local hemostatic agents
  - Antifibrinolytic agent (tranexamic acid) (the only effective treatment of patients with FV Quebec disorder)
  - Hormonal control of menses (oral contraceptives)
  - Iron supportation (if necessary)
- Corticosteroids are not beneficial.
- Some patients will require stem cell transplantation.
- **Avoid drugs with antiplatelet effect!**