Platelets and Platelet Disorders

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Platelets: Function and Dysfunction

- Production and life span
- Morphology and function
- Qualitative disorders
- Quantitative disorders
Megakaryocyte and Platelet Development

Italiano & Hartwig, in Platelets by A. Michelson (2002)
Birth of a Platelet

*Italiano et al J Cell Bio 147(6) 1299-1312, 1999*
Platelet Shedding

Junt et al. Science. 2007; 317(5845):1767-1770
Regulation of Platelet Production

- Thrombopoietin (TPO) - primary regulator of megakaryocyte development and platelet number
- Thrombopoietin receptor, c-mpl, located on hematopoietic stem cells, megakaryocytes and platelets
- Major site of thrombopoietin production: liver cells
- May be regulated by desialation of aging platelets
- Thrombopoietin levels
  - ↑ in marrow failure states eg aplastic anemia
  - NI or slight ↓ in ITP, essential thrombocythemia
  - ↓ in liver failure
Platelet Turnover and Aging

• Average platelet lifespan: 7 – 10 days
• Removed from circulation by monocyte – macrophage system
• Approximately 25 – 35% of circulating platelets located in the spleen
• 15 – 25% of the daily turnover of all platelets utilized for maintenance of vascular integrity
• Young “reticulated” platelets
  – contain messenger RNA which can be detected by labeling with RNA fluorochromes such as thiazole orange
  – May be functionally more competent than old platelets
Platelet Survival in Normal Subject, ITP Patient and Patient with Hypersplenism

J.N. George, in Hemostasis and Thrombosis by Coleman et al (2001)
Platelets: Function and Dysfunction

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Platelet Anatomy

Resting Platelet  Activated Platelet

J. George  Lancet 2000 ; 355 :1531-1539
Platelet Architecture

• Shape: round and flat discs, with diameter of 1-2 µm and volume of 7-9 fL
• Plasma membrane
  – Receptor glycoproteins eg GPIIb / IIIa ( αIibβ3 )
  – Surface-connected canalicular system
• Cytoskeleton
• Organelles
  – Mitochondria, lysosomes, and peroxisomes
  – Alpha granules ( ~ 80 per platelet )
  – Dense granules ( 3 - 8 per platelet )
Platelet Granules

• Alpha Granules
  – β-thromboglobulin
  – PF-4
  – PDGF
  – TGF-β
  – VEGF
  – Fg
  – Many others

• Dense Granules
  – Serotonin
  – ATP, ADP and GDP, GTP
  – Histamine
Enumeration of Platelets

• **Normal platelet count**: 150-400 x 10^9/L
• **2.5% of “normals” will have platelet count** < 150 x 10^9/L

• **Electronic**
  – Not accurate at low counts (<10 x 10^9/L)
  – Does not detect megathrombocytes
  – Does not detect pseudothrombocytopenia

• **Peripheral blood smear**
  – Number (15,000 per mm^3/ platelet / high power field)
  – Size
  – Morphology (e.g. Gray platelet syndrome)
  – Other (e.g. schistocytes, leukocyte inclusions, leukemia)
Platelet Participation in Hemostasis

1. ROLLING/ADHESION / ACTIVATION
   Platelets bind to exposed vWF, collagen via the platelet GPIbα and GPVI receptors

2. AGGREGATION
   Platelets aggregate through cross-linking of platelet activated GPIIb / IIIa by fibrinogen or vWF

3. PROPOGATION OF THE COAGULATION REACTION
   Activated platelets provide an anionic aminophospholipid (PL) rich surface for the assembly of procoagulant enzyme complexes
   - Tissue factor – VIIa
   - Tenase (FIXa, FVIIIa, Ca++,PL)
   - Prothrombinase (FXa, FVa, Ca++, PL)

4. CLOT STABILIZATION
   FXIIIa
Hemostatic Plug Formation

A. Injury

B. Initiation

C. Extension

D. Stabilization

Thrombus formation in vivo

Clinical Manifestation of Platelet Disorders

• Primary hemostatic disorder
  – Bleeding into skin and mucous membranes
  – Petechiae, purpura, ecchymoses, oropharyngeal bleeding, epistaxis, gastrointestinal bleeding, hematuria, menorrhagia
  – Hemarthroses, intramuscular hematomas uncommon
  – Exceptions
    • vWD type 3 – carries FVIII, causes factor-type bleeding
    • FXI deficiency – causes platelet-type bleeding
Etiology of Thrombocytopenia

- **Decreased platelet production**
  - Congenital thrombocytopenias e.g. TAR, congenital amegakaryocytic thrombocytopenia (CAMT), Fanconi anemia
  - Aplastic anemia
  - Marrow infiltration e.g. acute leukemia
  - Myelodysplasias
  - Infection e.g. HIV
  - Drugs

- **Increased platelet destruction**
  - IMMUNE
    - ITP
    - Alloimmune thrombocytopenia
    - Infection
    - Drugs
  - NON-IMMUNE
    - Disseminated intravascular coagulation (DIC)
    - Kasabach-Merritt syndrome
    - TTP / HUS

- **Platelet sequestration**
  - Hypersplenism
Assessment of Platelet Function

• Bleeding time
• Platelet aggregation
  – Agonists: collagen, arachidonate, epinephrine, ADP, ristocetin
  – Glanzmann thrombasthenia (reduced / normal GPIIb/IIIa)
    • no response to collagen, arachidonate, epinephrine, ADP
    • response to ristocetin
  – Bernard-Soulier syndrome (reduced / normal GPIbα)
    • normal response to collagen, arachidonate, epinephrine, ADP
    • no response to ristocetin
• Automated platelet function tests e.g. PFA-100
• Platelet EM
  – Whole mount EM to measure dense granules
  – Transmission EM for alpha granules and general morphology
• Flow cytometry
  – Measure surface glycoprotein levels (approximately 80,000 copies of GP IIb/IIIa and 25,000 copies of GPIb-IX-V / resting platelet)
Platelet Aggregation Testing

- Use platelet rich plasma or whole blood
- Measure change in light transmission through platelet-rich plasma, or:
- Measure change in impedance in whole blood
- Use epinephrine, ADP, collagen, ristocetin, and arachidonic acid as agonists
- Problems with thrombocytopenia ( <100 x 10^9/L )
- Subject to artifact and technical variables
Platelet Aggregation Profiles of Normal Controls, Glanzmann Thrombasthenia & Bernard-Soulier Syndrome Patients

Shapiro AD  Haemophilia 2000 ; 6 (Suppl 1) : 120-127
## Typical Platelet Aggregation Response in Inherited Platelet Function Disorders

<table>
<thead>
<tr>
<th></th>
<th>STORAGE POOL DISEASE</th>
<th>GLANZMANN THROMBASTHENIA</th>
<th>BERNARD-SOULIER SYNDROME</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLLAGEN</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
</tr>
<tr>
<td>ADP</td>
<td>↓ ( secondary )</td>
<td>↓</td>
<td>N</td>
</tr>
<tr>
<td>EPINEPHRINE</td>
<td>↓ ( secondary )</td>
<td>↓</td>
<td>N</td>
</tr>
<tr>
<td>ARACIDONIC ACID</td>
<td>N</td>
<td>↓</td>
<td>N</td>
</tr>
<tr>
<td>RISTOCETIN</td>
<td>N</td>
<td>N</td>
<td>↓</td>
</tr>
</tbody>
</table>
Function = Pathways

Platelets: Function and Dysfunction

- Production and life-span
- Morphology and function
- Qualitative disorders
- Quantitative disorders
Glanzmann Thrombasthenia

- Autosomal recessive inheritance (high rate of consanguinity)
- Severe mucocutaneous bleeding starting in infancy
- Deficiency or abnormality of GPIIb/IIIa (platelet $\alpha$IIb$\beta$3 integrin)
- Normal platelet count and morphology
- Absent platelet aggregation in response to ADP, epinephrine, collagen
- Normal ristocetin-induced platelet agglutination
- Treatment: local measures, DDAVP, fibrinolytic inhibitors, platelet transfusion, FVIIa
Bernard-Soulier Syndrome

- Autosomal recessive inheritance (consanguinity frequent)
- Deficiency or abnormality of GPIbα, GPIbβ, GPIX
- Prolonged bleeding time
- Normal platelet aggregation in response to ADP, epinephrine, and collagen
- Abnormal or absent agglutination in response to ristocetin
Bernard-Soulier Syndrome
Milder Inherited Platelet Defects

• Sites of defects
  – Membrane receptor (collagen, ADP or thromboxane)
  – Signal transduction apparatus
  – Prostaglandin generation mechanism (cyclo-oxygenase or thromboxane synthetase)
  – Storage granules (“storage pool disease”)

• Clinical features
  – Mild mucocutaneous bleeding
  – Variable and often ill-defined inheritance patterns
  – Some disorders feature other abnormalities
    • Hermansky-Pudlak (occulocutaneous albinism)
    • Chediak-Higashi syndrome
Milder Inherited Platelet Defects

- Normal platelet count and morphology by light microscopy
- PFA-100 closure time abnormal with collagen/epinephrine cartridge but often normal with collagen / ADP
- Defective but not absent platelet aggregation in response to ADP, epinephrine and collagen (reduced or absent second wave)
- Abnormal platelet EM and platelet ATP secretion in storage pool disease (dense granule deficiency)
Gray Platelet Syndrome

- Autosomal recessive (dominant in 1 family)
- $\alpha$-granule storage pool deficiency
- Mild bleeding disorder
- Bleeding time normal-prolonged
- Large, agranular, gray, platelets
- Severe deficiency of $\alpha$-granule proteins
Gray Platelet Syndrome
Storage Pool Deficiency

DENSE GRANULE DEFICIENCY

• Seen only by electron microscopy (whole mount)
• Granules contain serotonin, calcium, ATP (non-metabolic pool) and ADP
• Absence leads to impaired secondary aggregation
• Decreased platelet serotonin and increased ATP/ADP ratio
• Primary inherited disorder or secondary to Chediak-Higashi or Hermansky-Pudlak syndromes
Defects in the Interaction Between von Willebrand Factor & Platelet Surface GPIb/IX/V

- Loss of GPIb/IX/V
  - Bernard-Soulier syndrome
- Gain of function (enhanced interaction between GPIb/IX/V and VWF)
  - Type 2B von Willebrand disease (VWF mutation)
  - Platelet-type von Willebrand disease (“Pseudo” VWD) (GPIbα mutation)
  - Clinical picture:
    - Thrombocytopenia, especially with pregnancy and stress
    - Variably reduced VWF level
    - Increased ristocetin-induced platelet aggregation (using 0.5 mg/ml ristocetin)
MYH9-related Disease

- Autosomal dominant
- Gene: Nonmuscle heavy chain gene 9 (MYH9)
- Protein: Nonmuscle myosin heavy chain IIA

May-Hegglin anomaly

Drachman JG  Blood 2004 ;1003 : 390-398
# MYH9-related Disease

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>May-Hegglin Anomaly</th>
<th>Sebastian Syndrome</th>
<th>Fechtner Syndrome</th>
<th>Epstein Syndrome</th>
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<tbody>
<tr>
<td>Macrothrombocytopenia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Leukocyte-inclusions (Döhle-like bodies)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hearing impairment</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cataract</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Nephritis</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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Seri M et al Medicine 2003; 82: 203-215
N. Pujol-Moix et al Haematologica 2004; 89: 330-337
Acquired States of Platelet Dysfunction During Childhood

- Infection
- Liver disease
- Renal failure
- Cardiac disease
  - Cyanotic congenital CHD
  - Post-cardiopulmonary bypass
- Malignancy
- Drugs
Drugs and Platelet Dysfunction in Pediatric Patients

• Aspirin (irreversible inhibition of cyclo-oxygenase)
• Other non-steroidal anti-inflammatory agents
  – How serious?
• Valproic acid
• Semi-synthetic penicillin derivatives
• Psychotropic drugs
• Herbal – garlic, ginko, ginseng (in pharmacological doses)
Treatment of Qualitative Platelet Defects

- Remove or discontinue offending agent (e.g. drug)
- Treat underlying disease (e.g. dialysis)
- Local measures (pressure, Gelfoam, desiccated collagen, etc.)
- Amicar
- Desmopressin (DDAVP)
- RBC transfusion if patient anemic
- Recombinant Factor VIIa
- Platelet transfusion
Platelets: Function and Dysfunction

- Production and life-span
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- Quantitative disorders
# Classification of Inherited Thrombocytopenia According to Platelet Size

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<tr>
<th>DISORDER</th>
<th>GENE</th>
<th>INHERITANCE</th>
</tr>
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<tbody>
<tr>
<td><strong>SMALL PLATELETS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiskott Aldrich syndrome</td>
<td>WAS</td>
<td>X-linked</td>
</tr>
<tr>
<td>X-linked thrombocytopenia</td>
<td>WAS</td>
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<tbody>
<tr>
<td><strong>NORMAL-SIZED PLATELETS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Thrombocytopenia absent radius (TAR)</td>
<td>RBM8A</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>- Congenital amegakaryocytic thrombocytopenia (CAMT)</td>
<td>C-mpl</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>- Congenital amegakaryocytic thrombocytopenia with radioulnar synostosis</td>
<td>HOXA11</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>- Familial platelet disorder with predisposition to acute myelogenous leukemia</td>
<td>AMLI</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>- Familial platelet disorders</td>
<td>RUNX1</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>DISORDER</td>
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<td>ASSOCIATED ABNORMALITIES</td>
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<tr>
<td>----------------------------------------------</td>
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<td>----------------------------------------------</td>
</tr>
<tr>
<td>BERNARD - SOULIER syndrome</td>
<td>GPIbα, GPIbβ, GPIX</td>
<td></td>
</tr>
<tr>
<td>Velocardo-facial syndrome</td>
<td>GPIbβ (22q11)</td>
<td>Cleft palate, cardiac defects, learning disabilities</td>
</tr>
<tr>
<td>Pseudo von Willebrand disease</td>
<td>GPIbα</td>
<td></td>
</tr>
<tr>
<td>X-linked thrombocytopenia and dyserythropoiesis ± anemia</td>
<td>GATA1</td>
<td></td>
</tr>
<tr>
<td>MYH9 – related disease</td>
<td>MYH9</td>
<td>Leukocyte inclusions, high tone hearing loss, renal involvement, cataracts</td>
</tr>
<tr>
<td>Paris – Trouseau type thrombocytopenia</td>
<td>FLI1</td>
<td></td>
</tr>
<tr>
<td>Jacobsen’s syndrome</td>
<td>FLI1</td>
<td>Cardiac defects, mental retardation</td>
</tr>
<tr>
<td>Gray platelet syndrome</td>
<td>NBEAL2</td>
<td></td>
</tr>
<tr>
<td>Mediterranean Macrothrombocytopenia</td>
<td>ABCG5/ABCG8</td>
<td></td>
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</table>
Suspected platelet disorder

CBC, smear review

Normal PLT count

Platelet function studies: Abnormal
- Risto only: BS
- ADP: P2Y defect
- All agonists except Risto: GT

VWD studies: Abnormal
- Plt type VWD, VWD 2B

Non-specific: other FPD

No secondary wave: secretion defect, HP, GPS, QPS

Low PLT count

Small PLT
- WAS

Large PLT
- BS, MYH9, GPS

Normal size
- CAMT, TAR, GATA-I, other FPD

Platelet Defect Algorithm
Guidelines for Prophylactic Platelet Transfusion

- Platelet count <10 x 10^3 / μL in children
- Platelet count <20 x 10^3 / μL in febrile children
- Platelet count <30 x 10^3 / μL in neonate
- Platelet count <50 x 10^3 / μL in stable premature infant
- Higher (<100 x 10^3 / μL) in bleeding or pre-op premature infant
- Platelet count <50 for spinal tap, <80 – 100 for neurosurgery
Guidelines for Platelet Transfusion in Patients with any Platelet Count

• active bleeding in association with a qualitative platelet defect or decreased platelet count

• unexplained, excessive bleeding in a patient undergoing cardiopulmonary bypass

• patient undergoing ECMO:
  – with platelet count of < 100 x 10^3 / μL or with higher platelet counts and bleeding
Platelets and Platelet Disorders

Questions?

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