HEMOSTASIS: A system to control blood loss from spontaneous or traumatic breaks in the blood vessel.

THROMBOSIS: The formation of a blood clot within a blood vessel.
Concept: A Balance

Hemostasis

• Outside of a vessel
• Physiological

Thrombosis

• Within a vessel
• Pathological (abnormal)
Concept: Types of Clots

Arterial Clots
• Fast flowing arteries
• Platelet rich

Venous Clots
• Slow flowing veins
• Fibrin/ red-cells rich
**SITE**

Vein
(slow blood flow)

Artery
(fast blood flow)

**TREATMENT**

Anticoagulant
Heparin
Coumadin

Anti-platelet agents
Platelets

Structure / Function

Role in Hemostasis / Thrombosis

Approach to Thrombocytopenia

Thrombocytopenia examples
Platelets: Overview

- Circulating, Anuclear blood cells

- Closest relative WBC
  » share functions
  » share antigens

- Originate from megakaryocytes (in turn stem cells)

- Live 7 – 10 days, clearance senescence
Platelet Genesis

Stem Cells (CD 35+)

WBC Precursors

Lymphocytes

Red Blood Cells

Megakaryocytes

Monocytes

Platelets

Thrombopoietin
Platelet Formation

Thrombopoietin

(Constitutively Secreted)

c-mpl

Platelets

Clearance

Megakaryocytes

Proliferation
Platelet Anatomy: Surface:
Internal

- Carbohydrate Rich Glycocalyx
- Negative Charge
  P.P.L (esp P.S)
  Clotting Function
- Typical Bilipid Layer
- Surface Connected tubular (canalicular) System
- Cytoskeletal System Including Actin-Binding Protein
ANATOMY OF A PLATELET

Dense granules
ADP
Serotonin
Aggregation

alpha granules
Fibrinogen
vWF
V
Clotting

BTG
PF-4
PDGF
Platelet-specific
components

Albumin
IgG
HMWK

Mitochondria

Bilipid layer

Note: Platelets lack a nucleus and irreversibly inactivated enzymes are not regenerated during the platelet's 7 to 10 day lifespan
Platelet Participation in Hemostasis

Adhesion:
- GP Ib (passive)
- VWF (activated by shear)
- Almost impossible to inhibit (medically)

Aggregation: Secretion
- GP IIb/ IIIa (activated)
- Fibrinogen (RGD) (passive)
- Can be inhibited

Coagulation
- Site of assembly of coagulation factors
- Hard to inhibit
Platelet Glycoproteins

Step 1: Isolate Membrane

Step 2: Dissolve in detergent

Step 3: Separate by size

\[
\begin{array}{cc}
\text{Largest} & I \\
\uparrow & \\
\text{Smallest} & X
\end{array}
\]
PLATELET

ENDOTHELIAL CELL
Platelet Adhesion

Primarily: GP Ib/IX (V)

Secondarily: GP Ia/ IIa

Ligands: VWF, Collagen

Deficiency: Bernard-Soulier (AR)
PLATELET ADHESION

Thrombin and vWF binding region

GP Ib
vWF
Endothelial Cell

GP Ib

Glycoprotein Ib
Post Adhesion Events

PLATELET ACTIVATION and SECRETION:

30%,* Prostaglandin Pathway
30% Direct Granular Release (ADP)
30% Unknown

* rough estimates
Prostaglandin Pathway of Platelets

ACTIVATION

↑ Ca ++

Phospholipase A-2

Arachidonic Acid (AA)

PG:G₂

PG:H₂

TX:A₂

PG D₂, E₂ (Inactive)

G- Protein

Cyclo oxygenase

ASA

(Thromboxane A₂)

Vasoconstrictor

Platelet Activation
Platelet Activators

Strength

- ADP ++
- Collagen +++
- TXA₂ ++
- Thrombin ++++
- Adrenalin +

G Proteins → ↑ Ca²⁺
Platelet Aggregation

- GP IIb/IIIa  (25,000 – 50,000 surface, 50,000 – 100,000 total)
- Once activated GP IIb/IIIa binds fibrillar molecules at arginine (R), glycine (G), aspartate (D)
  - Fibrinogen  ++++
  - Fibronectin  ++
  - Von Willebrand ++
- GP IIb/IIIa Deficiency - Glanzmann’s (AR)
Integrins

Molecules Important for Cell Adhesion

2 chains (heterodimers) $\alpha, \beta$

Platelet integrins

<table>
<thead>
<tr>
<th>$\alpha_2 \beta_1$</th>
<th>(GP Ia / IIa)</th>
<th>Binds</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_5 \beta_1$</td>
<td>(GP Ic / IIa)</td>
<td>Binds</td>
<td>Fibronectin</td>
</tr>
<tr>
<td>$\alpha_{IIb} \beta_3$</td>
<td>(GP IIb/ IIIa)</td>
<td>Binds</td>
<td>Fibrinogen</td>
</tr>
</tbody>
</table>
INTEGRINS

- OFTEN CELL-SPECIFIC
- CELL “RESPONSIVE” (need activation)
- ALL RECOGNIZE ARG-GLY-ASP (RGD sequence)

EXTRACELLULAR domain

CYTOPLASMIC domain

TRANSMIT SIGNALS
ACTION OF PLATELETS

Immediate: Plug the damaged vessel wall

Long Term: Promote vessel wall repair
PATHWAYS OF PLATELET INHIBITION

Adhesion

Aggregation

Pathway Inhibition

INHIBITORS

Cyclooxygenase Receptors
- Thrombin
- Collagen
- ADP
## Antiplatelet Agents

<table>
<thead>
<tr>
<th>AGENT</th>
<th>SITE</th>
<th>EFFECTIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA</td>
<td>C.O.</td>
<td>About 20% R.R.R. (CAD; T.I.A)</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>ADP-inhib.</td>
<td>Perhaps more effective than ASA</td>
</tr>
<tr>
<td>GP IIb/IIIa Inhib.</td>
<td></td>
<td>Effective, but cause bleeding.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Currently used for PCI</td>
</tr>
</tbody>
</table>
Low Platelets (any cytopenia)

UNDERPRODUCTION

Increased Destruction
- Non immune (D.I.C.)
- Immune

Sequestration
- Hypersplenism

Dilution
- Post Operative
THROMBOCYTOPENIA

1. Failure of Production
2. Reduced Survival
3. Increased Pooling
Platelet Underproduction

- Almost always all cell lines (RBC, WBC) reduced

- Primary - (marrow failure – aplastic anemia)

- Secondary - chemotherapy
  immune aplasia
<table>
<thead>
<tr>
<th>CAUSE</th>
<th>PERIPHERAL BLOOD FINDINGS</th>
<th>BONE MARROW MEGAKARYOCYTES</th>
<th>CLINICAL EXAMPLE</th>
</tr>
</thead>
</table>
| Thrombocytopenia due to an increased rate platelet destruction | Isolated thrombocytopenia | Normal to increased numbers. Megakaryocytes have increased numbers of nuclei | A) Immune ITP  
B) Non-immune DIC |
| Thrombocytopenia due to sequestration of the platelets in the spleen | Thrombocytopenia plus leukopenia plus a variable degree of anemia | Normal | Hypersplenism |
| Thrombocytopenia due to underproduction       | Isolated thrombocytopenia is rare except for amegakaryocytic | Reduced number of megakaryocytes | A) Chemotherapy  
B) Amegakaryocytic thrombocytopenia |
| Thrombocytopenia due to hemodilution          | Thrombocytopenia plus anemia plus a variable degree of leukopenia | Normal | Hemodilution from cardiopulmonary bypass |
Platelet Sequestration

- Sometimes all cell lines
- Almost always spleen enlarged
- Moderate thrombocytopenia (40 – 100 x 10^9/L)
Platelet Destruction

- Almost always only platelets reduced

- Non immune D.I.C.
  Infection

- Immune Drug - Heparin
  - Quinine/ Quinidine

  Infection - Bacteria
  - H.I.V.

  Idiopathic - I.T.P.
THROMBOCYTOPENIA

BLEEDING

THROMBOSIS
- TTP / HUS
- HIT

OUTCOME

NOTHING

TRIVIAL

FATAL
MANAGEMENT OF A THROMBOCYTOPENIC PATIENT

1. Estimate the bleeding risk

2. Determine the cause of the thrombocytopenia.
   (a) Underproduction
   (b) Increased destruction
   (c) Sequestration
Idiopathic Thrombocytopenic Purpura (ITP)

- Very common (? 1/1,000) autoimmune disorder
- Children ↔ elderly, often women, age 20 – 40
- Mild to severe thrombocytopenia
- Splenectomy often (2/3) curative
Idiopathic Thrombocytopenic Purpura

Determine the risk:
- History (duration)
- Severity of hemostatic impairment

Exclude other causes:
- Viral (HIV, hepatitis, mono)
- Immunological (SLE)
- Other (sarcoid, lymphoproliferative)

Decide if treatment is required:
Platelet Destruction within Vessels

Heparin-induced thrombocytopenia (HIT)

Thrombotic thrombocytopenic purpura (TTP)

Hemolytic Uremic Syndrome (HUS)
Thrombotic Thrombocytopenic Purpura

PENTAD
1. Schistocytic Hemolytic Anemia
   (Fragmentation Hemolysis)
2. Thrombocytopenia
3. Acute Neurological Events
4. Renal impairment
5. Fever
Thrombotic Thrombocytopenic Purpura

Untreated

80 – 90% Mortality

Treated

10 – 20% Mortality
THROMBOTIC THROMBOCYTOPENIC PURPURA

**DIAGNOSIS:**

1. Thrombocytopenia 100% * 24
2. Schistocytic Hemolytic Anemia 100% 90
3. Neurological Events 65%
4. Fever 25%
5. Renal Impairment 50%

* Canadian Trial (NJEM 1991)
Hemolytic Uremic Syndrome

1. Schistocytic Hemolytic Anemia
   (Fragmentation Hemolysis)

2. Thrombocytopenia

3. Renal Impairment
## Hemolytic Uremic Syndrome

<table>
<thead>
<tr>
<th>Type</th>
<th>Source</th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EPIDEMICS</strong></td>
<td>(infectious)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Children</td>
<td>good</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>poor</td>
<td></td>
</tr>
<tr>
<td><strong>ENDEMIC</strong></td>
<td>(non-infectious)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>poor</td>
<td></td>
</tr>
</tbody>
</table>
HEPARIN-INDUCED THROMBOCYTOPENIA (HIT)

Update:

- The most important and catastrophic complication of drug therapy
- Every year results in many tens of thousands of serious complications
- The impact of HIT is becoming increasingly important
- Effective treatments are now available
TYPICAL PRESENTATION OF HIT

- Moderate (m=40,000) thrombocytopenia that onsets five or more days after starting heparin

- Bleeding rare

- Thrombotic complications – can present simultaneously or subsequently

- Thrombi – Venous:Arterial 4:1
Heparin-Induced Thrombocytopenia

Pathophysiology: IgG/Heparin Immune Complexes activated platelets producing procoagulant microparticles

Diagnosis: Clinical, confirm with serology

Treatment: Stop Heparin, initiate thrombin inhibitor (hirudin, argatroban)

Outcome: 20% mortality