I. INTRODUCTION

In the previous lecture we reviewed the soluble coagulation factors, "limiting reactions", anticoagulants, common coagulation syndromes and fibrinolysis. We will now review the cellular aspects of the coagulation system, namely, endothelial cells and platelets. Most of our time will be devoted to platelets since relatively little is known about the participation of the endothelium in most disease processes. Endothelial cell biology, however, is the most intensely studied area of coagulation research today and can be expected to provide many new insights in the coming years.

II. THE ENDOTHELIUM

A. Extensive organ lining all of our blood vessels

1. Surface area that of a football field
2. Most endothelial cells are in capillaries
3. Properties vary from organ to organ, i.e. fenestrated vs non-fenestrated endothelium.

B. Biochemical properties

- **non-thrombogenic state**
  - **major surface membrane components**
    - Heparan sulfate proteoglycan
    - Thrombomodulin (decreased by IL1, TNF, homocysteine)
    - Plasminogen binding site (converts plasminogen to plasmin)
  - **major metabolites/factors secreted**
    - PGI2 (prostacyclin) - inhibits platelet activation
    - Adenosine
    - ADPase
    - Endothelium-derived relaxation factor (EDRF=NO2)
Tissue plasminogen activator (TPA)

thrombogenic ("activated") state

major surface membrane components
Factor IX, X binding sites
Tissue factor (increased by IL1, TNF)
Ligands for WBC receptors CD11/CD18 - mediate
binding of WBC to endothelium:
- ICAM I
- ELAM I (increased by IL1, TNF)
Platelet activating factor (PAF)
major metabolites/factors secreted
von Willebrand factor
leukotrienes
endothelin (contracts smooth muscle cells)
Platelet activating factor inhibitors (PAI) (increased by
IL1, TNF)
O2⁻ (inactivates EDRF)
Platelet-derived growth factor

C. Concept: **non-thrombogenic endothelium becomes thrombogenic endothelium**

1. Cytokines [interleukin 1 (IL1) and tumour necrosis factor (TNF)] cause non-thrombogenic endothelium (normal TM and TPA, low TF and PAI) to become thrombogenic endothelium (low TM and TPA, elevated TF and PAI)

2. Change in balance between non-thrombogenic and thrombogenic properties of endothelium may explain pathology of some thrombotic diseases associated
with inflammatory states (i.e., SLE)

D. Known Diseases of the Endothelium
- von Willebrand’s Disease
- Hypercoagulable states due to decreased release of TPA
- Hypercoagulable state and premature atherosclerosis in homocysteinuria (diminished production of Ca)

Atherosclerosis
Tumour metastasis
Hypercoagulable states

III. PLATELET STRUCTURE

A. Surface membrane - deeply invaginated open canalicular system - makes for rapid platelet activation
- ABO blood group antigens
- Class I (but not Class II) HLA antigens
- Fc receptor
- Agonist receptors: epinephrine, collagen (GPVI), ADP (P2Y12), thrombin (PAR-1)
- Glycoprotein Ib (GPIb) - binding site for VWF - deficient in Bernard-Soulier Syndrome
- Glycoprotein IIb/IIIa (GPIIb/IIIa) - heterodimer in integrin family of surface membrane adhesive protein receptors. Major binding site for fibrinogen (and also fibronectin, vitronectin and VWF) via common R-G-D (arg-gly-aspartic acid) sequence. This binding is increased upon platelet activation. Occupies 10-20% of surface area of platelet. Major antigen target in ITP. Absent in Glanzmann's thrombasthenia.

B. Platelet cytoskeleton - discoid shape in circulation converts to a sphere with filopodia upon platelet activation.
- Circumferential ring of microtubules
- Actin filaments bind to cytoplasmic side of IIb/IIIa

C. Granules - released upon platelet activation
- Lysosomes - hydrolytic enzymes may mediate inflammatory reactions
- Dense granules - avg 10/platelet - release of these granules is essential for platelet aggregation
- Alpha granules - avg 100/platelet - multiple cytokines. P-selectin [CD62, PADGEM (Platelet Activation-Dependent Granule External Membrane)] - 140 kd protein on inner
surface of $\alpha$ granule becomes exposed on external cell membrane upon activation.


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<th>dense granules</th>
<th>lysosomal granules</th>
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<tr>
<td>Plasma proteins</td>
<td>ATP</td>
<td>Acid hydrolases</td>
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<td>$\alpha_1$-anti-trypsin</td>
<td>ADP</td>
<td>$\beta$-galactosidase</td>
</tr>
<tr>
<td>$\alpha_2$-macroglobulin</td>
<td>GTP</td>
<td>$\beta$-glucuronidase</td>
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<td>C1-inhibitor</td>
<td>serotonin</td>
<td>heparitinase</td>
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<tr>
<td></td>
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<td>collagenase</td>
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Coagulation proteins
- Factor XI (or XI-like)
- Factor V
- Fibrinogen
- VWF

Heparin-binding proteins
- Thromboglobulin
- PF-4

Thrombospondin

Fibronectin

Growth factors
- TGF-$\beta$ (Transforming growth factor $\beta$)
- PDGF (Platelet-derived growth factor)
- EGF (Epidermal growth factor)

IV. PRODUCTION OF PLATELETS

A. Precursor cell in bone marrow is **megakaryocyte** *(Kuter 2001)*
   1. Uncommon cell in bone marrow – 0.1% of all cells
   2. Massive cell - 50 micron diameter (vs 5 micron for RBC) - each produces 1000 - 2000 platelets
   3. Polyploid nucleus - on average 16 haploid units (N) of DNA

B. Pluripotential stem cell gives rise to megakaryocyte colony forming cell (Meg-CFC), a replicating megakaryocyte precursor cell. At some stage Meg-CFC stops dividing cytoplasm but continues to replicate DNA giving rise to polyploid megakaryocyte.

C. Platelet formation - mature megakaryocyte migrates to bone marrow sinusoid [under SDF-1 signal (Avecilla, *et al* 2004)] where platelets are formed via
   1. Extension of pseudopods into sinusoid and budding off of
proplatelets (Italiano, et al 1999)

OR

2. Migration of megakaryocyte or large portions of its cytoplasm into sinusoid; circulation to lungs where it is made into platelets. Very recent data suggests that lung may account for ~50% of proplatelet-platelet formation (Lefrancais, et al 2017).

D. Regulation of platelet production - principles
   1. Platelet count tightly regulated over life for any individual.
   2. Unlike normal hematocrit (which varies little in general population except between sexes), platelet count highly variable (150,000-400,000) between individuals.
   3. Platelet size inversely related to platelet count
   4. Body regulates platelet mass, not platelet count. Individuals with high normal platelet counts have small platelets; those with low normal platelet counts have larger platelets. Splenic sequestration does not alter platelet mass but the count decreases proportional to the increase in spleen size.
   5. Response to decrease in circulating platelet mass: increased number of megakaryocytes, increased size of megakaryocytes, increased megakaryocyte ploidy. This provides increased platelet production.

E. Thrombopoietin (TPO) is a cytokine that controls platelet production. Site of production is liver. Concentrations of TPO rise when platelet production is low and fall when platelet production is high (Bartley, et al 1994, de Sauvage, et al 1994, Kuter 1996a, Kuter 1996b, Kuter 2000, Kuter 2008, Kuter 2009a,
V. PLATELET FUNCTION (Kuter 1991)

A. **Vascular integrity** - in thrombocytopenic patients endothelial cells become thin with increased fenestrations (Kitchens 1977, Kitchens and Weiss 1975)

B. **Provide solid phase support for soluble coagulation factors**
   1. Phospholipid
   2. Xa, Va binding sites
   3. Protect activated factors (Xa, Va) from inhibitory proteins/limiting reactions

C. **Form 1° Hemostatic Plug** (Zucker and Nachmias 1985)
   1. Mediated by platelet activation - complex process whereby relatively inert discoid cell full of granules is converted into an adherent, spherical cell with many projections and with release of granules.
   2. Cellular steps in platelet activation
      - **adhesion** - platelet binds to exposed collagen via collagen receptor (GPVI) and is activated; mediated and stabilized by VWF binding to Ib platelet receptor
      - **granule release (secretion, degranulation)** - alpha, dense and lysosomal granules released [mediated by thromboxane A2 (TXA2) synthesis]
      - **recruitment** - release of ADP "activates" other platelets circulating nearby via the ADP receptor (P2Y12 receptor) and these activated platelets “stick” to the initial adherent activated platelets. Thrombin activates PAR-1 receptor
      - **aggregation** – circulating ADP-activated platelets “stick” to the initial adherent platelets. Fibrinogen and IIb/IIIa receptor are required for aggregation to occur.
   3. VWF stabilizes platelet binding to subendothelium. Increased bleeding tendency if:
a. VWF decreased in amount or function (von Willebrand's Disease)
b. Ib receptor absent (Bernard Soulier Disease)
c. Therapeutic infusion of experimental monoclonal antibody to Ib receptor (prevents adhesion)
d. Abnormal subendothelial collagen or other such proteins (prevents adhesion; such as may occur in osteogenesis imperfecta?)

4. Prostaglandin synthesis

a. Phospholipase A2 (PLA2) activated with platelet activation
b. In platelet produces \( \text{TXA}_2 \) - potent platelet agonist which causes granule release
   In endothelial cell produces \( \text{PGI}_2 \) - potent platelet antagonist that inhibits granule release
c. Inhibitors of platelet activation
   PLA2 inhibitors
cyclooxygenase inhibitors - ASA, NSAIDS, sulfinpyrazone
thromboxane synthetase inhibitors
\( \text{TXA}_2 \) competitors
\( \text{PGI}_2 \) - increases adenylate cyclase (increases cAMP)
   inhibits PLA2 activity and granule release
phosphodiesterase inhibitors - dipyridamole -
   increase cAMP and inhibit granule release
5. **Role of ADP and its receptor(s).** Platelets can be activated by multiple agonists, including collagen, ADP, and thrombin. The initial platelet monolayer adheres to and is activated by collagen. Upon release, the ADP enters the circulation and binds the ADP receptor (P2Y12) on nearby circulating platelets, activates them, and recruits them to the initial platelet layer. One result of platelet activation by collagen surfaces or ADP is a conformational change in the IIb/IIIa receptor. The conformational change in the IIb/IIIa receptor now allows the platelets in the initial monolayer to bind via fibrinogen with those recruited from the circulation. With the aggregation of additional platelets the primary hemostatic plug is formed.

   a. Ticlopidine, clopidogrel, prasugrel, ticagrelor (cangrelor did not get FDA approval) block ADP receptor (P2Y12)
   b. Prevent recruitment

6. **Role of thrombin and its receptor.** Thrombin generation by the coagulation cascade binds to the platelet protease-activated receptor-1 (PAR-1) and cleaves off a part of this receptor; once cleaved, the remaining
extracellular tail ("tethered ligand") binds to the receptor and activates the receptor.

a. Vorapaxar is a recently FDA-approved inhibitor of this mechanism. It was hoped that this inhibitor might reduce thrombosis without exacerbating bleeding; that is not the case
b. Vorapaxar prevents recruitment

7. Role of fibrinogen in platelet aggregation

![Diagram of platelet and fibrinogen interaction]

a. Upon platelet activation IIb/IIIa receptor undergoes conformational change
b. Binds fibrinogen (via fibrinogen R-G-D sequence)
c. Platelets aggregate
d. IIb/IIIa receptor missing in Glanzmann's thrombasthenia - severe bleeding disorder
e. Therapeutic infusion of monoclonal antibody (ReoPro®, abciximab) to fibrinogen binding site of IIb/IIIa blocks aggregation
f. Therapeutic infusion of short fibrinogen peptides (Integrillin®, eptifibatide) or non-peptides (Aggrastat®, tirofiban) blocks IIb/IIIa and prevents aggregation

7. Limiting reactions
   a. Blood flow
   b. Plasma and platelet ADPase
   c. PGI2

VI. PLATELET TESTS

A. Platelet count
1. Normal 150,000 - 350,000
   thrombocytopenia if <100,000
   thrombocytosis if > 450,000
2. Accurate count to +/- 3% at 10,000 - 3,000,000
3. Risk of bleeding (if qualitatively normal)
   50,000 - 100,000 - normal hemostasis
   20,000 - 50,000 - small increased risk
   < 5,000 - increased risk spontaneous hemorrhage
   Exception: At comparable low platelet counts, bleeding risk in ITP is much less than in leukemic patients.
   Platelets in ITP are young and usually large and hyperfunctional.
4. PLADO study (Slichter, et al 2010) shows relation of platelet count and bleeding

![Graph showing relation of platelet count and bleeding](image)

B. Bleeding time
   1. 4 - 9 minutes normal
   2. Prolonged (and inaccurate) if platelet count < 100,000
      (Harker and Slichter 1972)
   3. Screening test for qualitative abnormalities
   4. Poor (probably worthless) test to predict clinical bleeding.
      Except, perhaps, to predict bleeding in uremic patients undergoing renal biopsy. See (Rodgers and Levin 1990).
   5. Please don’t ever order!!!!

C. Platelet function tests - rarely helpful

VII. PLATELET DISORDERS - THROMBOCYTOSIS (Platelets > 450,000)

A. Post-splenectomy

B. Reactive - usually < 1,000,000
   1. Fe deficiency
2. Inflammation
3. Malignancy

C. Autonomous - essential thrombocythemia, P. vera, CML - platelets often > 1,000,000
   1. Often asymptomatic
   2. Risk of bleeding and/or thrombosis
   3. Treatment indicated if symptomatic


A. Causes

Artifact
Dilution
Sequestration
Decreased production
   metabolic: B12 deficiency, severe Fe deficiency
toxin: ethanol or drug exposure
congenital bone marrow abnormality:
   hypomegakaryocytic thrombocytopenia
      with absent radius
abnormal bone marrow: leukemia, aplastic anemia
   malignant infiltration
Increased consumption
non-immune: DIC, TTP, HUS
   hemangioma (Kasabach-Merritt)
   VWD IIb
   burns
immune:
   Fc-mediated (“innocent bystander”)
      drug related (like heparin)
      HIV related (via immune complexes).
   Fab-mediated
      neonatal isoimmune thrombocytopenia
      post-transfusion purpura
      immune (“idiopathic”) thrombocytopenic purpura (ITP)

B. Approach(Kuter 2013b)
   1. Review smear to exclude artifact due to clumping
   2. History to see if multiple transfusions (dilution)
   3. PE to assess for splenomegaly
   4. BM may be helpful to distinguish decreased production (decreased number, size and ploidy of megakaryocytes) from increased consumption (increased number, size and ploidy of megakaryocytes)
C. **Sequestration** - platelet count inversely proportional to spleen size. **Body maintains the platelet mass not the platelet count.** As spleen enlarges, mass remains constant but the platelet count falls.

![Diagram showing platelet count inversely proportional to spleen size](image)

D. **Thrombotic thrombocytopenic purpura (TTP)**

**Etiology:** Platelet activation with aggregates forming within vasculature of CNS, kidney and other organs. **Trigger** (infection ?) appears to decrease PG12 secretion from endothelial cells as well as increase PAF (platelet activating factor) on the surface of endothelial cells. This results in platelet activation, aggregation and thrombocytopenia but little or no fibrin deposition ("bland" platelet clots).

An alternative mechanism is that an antibody to VWF depolymerase (ADAMTS 13 protease) inactivates the protease and results in the appearance of unusually large VWF multimers that cause spontaneous platelet aggregation (Tsai 2004)

Narrowed lumen produces (a) ischemia of the affected vascular bed and (b) shearing of RBCs (forming schistocytes).

**Epidemiology:** May follow URI, shigella, E.coli O1857 (vero toxin producing), HIV infection or chemo. But most often no associated illness.

**Presentation:** pentad of findings - CNS symptoms (92%) ranging from headaches to stroke; thrombocytopenia (96%); microangiopathic hemolytic (MAHA) anemia (96%); fever (98%); renal dysfunction (88%). Pentad present in 70%. Triad (anemia, thrombocytopenia, neurologic symptoms) present in 90%.

**PE:** CNS defects
petechiae, purpura

**Lab:** Decreased platelets and hematocrit, increased
reticulocytes, elevated LDH, smear shows schistocytes and
decreased platelets, low haptoglobin, normal PT/PTT,
BUN/creat may be elevated. ADAMTS 13 protease activity low with or
without a concurrent inhibitor [see (Tsai 2004)].

Rx: Plasma exchange is primary therapy. Plasma infusion, steroids.
Follow symptoms and hemolysis parameters. Rituxan may
be indicated in relapsing patients with antibody to ADAMTS 13
protease.

Comment: If underlying process primarily affects renal
vasculature, the BUN/creat will be elevated (with +/-
CNS symptoms) and is referred to as adult hemolytic
uremic syndrome (adult HUS). Some authorities feel that in
adults HUS and TTP are simply variable manifestations of
the same disease process and prefer the term, thrombotic
microangiopathy, to describe both.

E. Drug-mediated thrombocytopenia
Etiology: Unlike autoimmune hemolytic anemia, most are
due to innocent bystander mechanism - platelet Fc
receptor binds antibody-drug complex and the opsonized
platelet is activated or cleared from the circulation.
Presentation: abrupt or gradual onset of thrombocytopenia,
may be severe (<10,000) or moderate (~50,000)
Rx: discontinue offending drug, IVIG; steroids rarely needed

Fc-MEDIATED PLATELET CONSUMPTION
("INNOCENT BYSTANDER MECHANISM")

F. HIV-related thrombocytopenia (Karpatkin 1988)
Incidence: Pancytopenia is frequently noted in HIV+
hemophiliacs, addicts and homosexuals.
But solitary thrombocytopenia seen in HIV+
patients often before other signs of disease
Etiology: Probably mediated in part by immune complexes
binding to Fc receptor of platelet with clearance of
these platelets by the spleen. (Megakaryocytes have also been shown to be HIV infected). A qualitative platelet defect may also be produced. An alternate hypothesis suggests that HIV-infected megakaryocytes undergo premature apoptosis and have ineffective thrombopoiesis.

**Laboratory:** Thrombocytopenia usually modest (~50,000) but may be severe (<10,000).

**Rx:** Treatment indicated only in symptomatic patients:

1. AZT (200 mg qid) and associate HIV therapy is primary treatment - 100% response rate in some studies. Platelet count may remain elevated even after stopping AZT. Anemia is common complication of therapy.

2. Other ITP treatments [steroids, splenectomy, IVIG, Winrho (infusions of anti-D antibody), azathioprine] also effective.

G. **Immune Thrombocytopenic Purpura** (ITP, formerly called *Idiopathic Thrombocytopenic Purpura*) [see (Cines and Blanchette 2002, Rodeghiero, et al 2009)]

**Incidence:** 1/10,000

**Etiology:** Polyclonal antibody to platelet antigen (determinant is usually part of IIb/IIIa complex). Antibody binds to platelet via its antigen-combining (Fab) region (note difference from drug and HIV-mediated thrombocytopenias). Cleared by spleen > liver. Most IgG, some IgM.(Harker and Finch 1969) Little evidence for intravascular lysis of platelets. Underlying immune disease (such as SLE) may be present, but usually is not. Platelets may also show qualitative abnormalities. Platelet production also inappropriately low.

(Gernsheimer, *et al* 1989, Kuter and Gernsheimer 2009)

[Diagram of platelet and antibody interaction]

**Fab-MEDIATED PLATELET CONSUMPTION**

(ITP)
Presentation: Hemorrhage, petechiae, bruising
Evaluation: Smear [careful evaluation to exclude spurious thrombocytopenia and to exclude schistocytes (seen in DIC, HUS and TTP)]; Bone marrow (to see if megakaryocytes increased); Coombs (to exclude concomitant autoimmune hemolytic anemia); retic, LDH, ANA, ESR.
Anti-platelet antibody testing rarely helpful and is not recommended. (Kelton 1983)

Natural history:
- Kids - 80% spontaneous remission in 6 - 12 months
- Adults - few remissions - highest estimate is 23%

Treatment - primary modalities:

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Treatment - secondary modalities:
- **Danazol** - decreases RE system Fc-mediated clearance
  (Schreiber, et al 1987)
- rituximab
- interferon
- cyclophosphamide, azathioprine (Figueroa, et al 1993)
- Winrho (anti-D antibody)
- Thrombopoietin [romiplostim (Nplate), eltrombopag (Promacta)]

IX. PLATELET DISORDERS - QUALITATIVE DEFECTS (Bellucci, et al 1983)

A. Overview

Defects in adhesion - VWD, infusion of antibody to Ib
Defects in granule release - storage pool disorders, ASA, dipyridamole, sulfinpyrazone
Defects in recruitment – ticlopidine/clopogrel/prasugrel/ticagrelor – ADP receptor inhibitors
Defects in aggregation - Glanzmann’s thrombasthenia, infusion of antibody to IIb/IIIa (ReoPro®), infusion of peptide (Integrelin®) or non-peptide (Aggrastat®) inhibitors of IIb/IIIa
B. von Willebrand Factor (VWF)

1. VWF synthesized as 230,000 MW protein in endothelial cells and megakaryocytes (platelets). Formed into polymers with MW up to 20,000,000. Constitutively released from endothelial cells - also stored in granules (Weibel-Palade bodies) in endothelial cells.

2. Functions:
   a. Only high MW polymers stabilize 1º hemostatic plug (make Bleeding Time normal)
   b. All MW sizes bind VIII and prolong its T1/2 - if VWF deficient, then VIII level decreases and PTT prolonged.

3. von Willebrand's Disease ("Classical" Type I)
   
   Etiology: VWF (of all MWs) made in endothelial cells but not released - hence circulating VWF present in low amounts - BT and PTT prolonged - bleeding
   
   Presentation: Lifelong, but variable, history of "platelet-type" bleeding (mucosal bleeding, epistaxis, bruising; rarely hemarthrosis)
   
   Lab: Elevated BT and PTT, low VIII level and VWF antigen
   
   Rx: DDAVP - releases preformed VWF in Weible-Palade bodies and is adequate for many bleeding episodes.(Mannucci 1986) Cryoprecipitate or purified VWF:VIII complex (Humate P) for severe episodes
   
   Recombinant VWF

4. "Severe Type III VWD" - No VWF synthesized - none stored in endothelial cells - responds only to VWF replacement with cryoprecipitate, VWF:VIII complex (Humate P), recombinant VWF

5. VWD-variant forms (Type II) - several other types of VWD in which VWF is made in adequate amounts but either fails to polymerize or makes polymers that are abnormal
   
   Rx – cryoprecipitate, VWF:VIII complex, or recombinant VWF

C. Bleeding disorder of uremia

   Etiology: unknown mechanism but platelet/endothelial interactions are felt to be abnormal and to cause prolongation of the BT
   
   Presentation: "Platelet-type" bleeding in patient with uremia
   
   Assessment: PT, PTT normal
   
   Extent of BT elevation may predict severity of bleeding risk
   
   Hematocrit
   
   Rx:
   a. Transfusion (or erythropoietin) to restore hematocrit
to > 30 - shortens BT and improves hemostasis in many with severe anemia

b. Plus any of the following modalities

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<th>Dose</th>
<th>Onset Effect</th>
<th>Peak Effect</th>
<th>Duration Effect</th>
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<td>DDAVP 0.3 µg/kg iv</td>
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<td>60 min</td>
<td>240 min</td>
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<tr>
<td>cryoppt 10 units iv</td>
<td>1 h</td>
<td>8-14 h</td>
<td>24 h</td>
</tr>
<tr>
<td>estrogens 0.6 mg/kg iv (conjugated) qd x 5</td>
<td>6 h</td>
<td>5 - 7 d</td>
<td>14 d</td>
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X. ANTIPLATELET AGENTS (Webster, et al 1990)

A. Overview (see (2011a, 2014a, 2014b) for excellent reviews)

  inhibition of adhesion - experimental

  inhibition of granule release -
  
  ASA - irreversible acetylation CYO
  NSAID - reversible inhibition CYO
  sulfinpyrazone – competitive inhibition CYO
  dipyridamole - ↑ intracellular cAMP

  inhibition of recruitment – ADP receptor inhibitors
  
  ticlopidine
clopidogrel
prasugrel
ticagrelor

  inhibition of recruitment – PAR-1 inhibitors
  
  vorapaxar

  inhibition of aggregation -
  
  ReoPro®
Integrylin®
Aggrastat®

LOW HCT NML HCT
B. Aspirin

Mechanism of action:
irreversible acetylation of cyclooxygenase
prevents release (and hence aggregation) but
not adhesion
most effective in preventing platelet
activation by biological, not artificial
surfaces

Pharmacology:
oral doses well absorbed
peak in 15-20 minutes
duration of effect: 300 mg has full effect for
48 h (until new platelets made)
platelets exposed to ASA are
inactivated for duration of
their lifespan (7 - 10 days)

Dose: 1 mg/kg/d inhibits both platelet and endothelial
cyclooxygenase
a. Meta analysis of 31 studies showed no difference
   in clinical effect of doses between 300 and 1500 mg/d
b. A single dose of 300 mg inhibits all TXA2 synthesis
   for 48 hours
c. 150 mg/d inhibits all TXA2 synthesis
d. Adding dipyridamole does not provide additional benefit

Side effects: proportional to dose

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<th></th>
<th>Placebo</th>
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<th>1200 mg/d</th>
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<td>GI bleeding</td>
<td>0.9%</td>
<td>1.5%</td>
<td>2.3%</td>
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<td>Minor GI problems</td>
<td>24%</td>
<td>29%</td>
<td>39%</td>
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Proven Benefits

TIAs
Secondary prevention of MI
Unstable angina
Saphenous vein bypass grafts
Following coronary angioplasty
Peripheral vascular disease
Migraines
Glomerulonephritis
Pregnancy

Unproven Benefits
Asymptomatic patients
? Non-valvular AF

No Proven Benefit

Valvular heart disease
Deep venous thrombosis

Contraindicated/Harmful

Thrombocytopenic patients

C. Dipyridamole

Mode of action:

1. ↑cAMP in platelets
   at high concentrations:
   inhibits phosphodiesterase which ↑cGMP
   and ↑platelet adenylate cyclase activity
   at physiologic concentrations:
   ↑plasma adenosine by ↓uptake by
   vasculature - indirectly leads to ↑
   platelet adenylate cyclase activity and ↑
   intracellular cAMP

2. Much better at preventing platelet binding to
   artificial surfaces than to biological surfaces

3. Vasodilatation

Pharmacology:

Highly variable oral absorption of immediate release form
-requires gastric acid
-markedly decreased in elderly with reduced gastric acidity
Does not potentiate warfarin effect

Newer modified release formulation: Aggrenox®: capsule contains 25
mg ASA tablet and 200 mg dipyridamole granules (extended
release granules containing dipyridamole and tartaric acid)
- improved absorption in patients with diminished gastric acidity

Clinical Use:

1. Three clinical studies show that it does not potentiate full dose
   ASA effect in secondary prevention of stroke

2. Little data to show any in vivo anti-thrombotic
   effect on biological surfaces

3. ↓platelet deposition on prosthetic grafts and during
   bypass surgery, thereby ↑platelet survival
4. Five studies in patients with prosthetic valves show that 350 - 400 mg/d added to warfarin ↓ embolic episodes without ↑ bleeding

5. However, modified release complex (ASA/dipyridamole, Aggrenox®) shown to be superior to ASA alone or Dipyridamole alone in secondary prevention of stroke (EPS 2 trial) – FDA approved

D. Sulfinpyrazone
   Mechanism of action:
   Competitive inhibitor of platelet cyclooxygenase
   More effective in preventing platelet binding to prosthetic than biological surfaces

   Pharmacology:
   Oral dose well absorbed
   Peak 1 - 2 h

   Clinical Uses:
   ↓ platelet consumption on prosthetic surfaces
   ↓ thrombosis of arterio-venous cannulae
   Nothing to support its use in other clinical conditions.

E. Ticlopidine (Ticlid®) – rarely used anymore
   Mechanism of action: inhibits platelet recruitment by multiple agonists, probably by preventing the ADP-mediated conformational change in the IIb/IIIa receptor, thereby preventing fibrinogen binding and subsequent aggregation

   Pharmacology: 24 - 48 h lag time before inhibiting platelets possibly due to need for metabolites to form or need to alter platelets at their formation from megakaryocyte
   Side effects: diarrhea, rash, neutropenia, TTP (rare)
   Dose: 250 mg bid
   Monitor WBC first two months
   Effect is irreversible - 7-10 days before platelets recover

   Clinical Uses: Few clinical studies
   Most so far show equivalency with aspirin in treating TIA, unstable angina and intermittent claudication.
Ticlopidine Aspirin Stroke Study - may be better than ASA in preventing recurrent stroke in females

Improves patency of saphenous vein bypass grafts in legs
(Becquemin 1997)

Because of expense, usually use instead of aspirin only in patients with aspirin "allergy" or GI intolerance

F. Clopidogrel (Plavix®)

Mechanism of action: like ticlopidine it inhibits platelet aggregation by irreversibly binding the platelet P2Y12 receptor and thereby preventing the conformational change in the IIb/IIIa receptor that is necessary for fibrinogen binding and subsequent platelet aggregation

Pharmacology: variably converted to active drug
24 - 48 h lag time before inhibiting platelets due to need for metabolites to form
Side effects: diarrhea, rash - unlike ticlopidine no increased risk of neutropenia but TTP has been reported (Bennett, et al 2000)
Dose: 75 mg/d; 300-600 mg loading dose
Effect is irreversible - 7-10 days before platelets recover

Clinical Uses: Rapidly increasing number of good clinical studies

CAPRIE Study: In patients with prior CVA, MI or PVD, 75 mg clopidogrel reduced risk of recurrent event slightly better than ASA (5.32% vs 5.83%, respectively) but no survival benefit (Bhatt, et al 2001, Jarvis and Simpson 2000)

CURE trial: clopidogrel plus ASA decreased cardiovascular deaths by 20% in patients with acute coronary syndromes compared with aspirin alone (cardiovascular events 11.5% with ASA and 9.3% with ASA plus clopidogrel). (Mitka 2001)

In patients after cardiac surgery, clopidogrel decreased subsequent cardiovascular events and caused less bleeding than did ASA (Bhatt, et al 2001)

Safer than ticlopidine and has replaced ticlopidine
Expensive substitute for ASA in secondary prevention of vascular events

Patient response highly variable

G. Prasugrel (Effient®)

**Mechanism of action:** like ticlopidine it inhibits platelet aggregation by irreversibly binding the platelet P2Y12 receptor and thereby preventing the conformational change in the IIb/IIIa receptor that is necessary for fibrinogen binding and subsequent platelet aggregation

**Pharmacology:** More efficiently converted to active drug than clopidogrel
More effective than clopidogrel
Increased risk bleeding, esp older pts
Not for patients with CVA/TIA, ≥75 unless high risk
Dose: 10 mg/d; loading dose (60 mg)
Effect irreversible: 7-10 days for platelet recovery

**Clinical Uses:**

In study of patients with ACS undergoing PCI, prasugrel plus ASA better than clopidogrel plus ASA in preventing MI and stent thrombosis(2009).
But higher rate of major, life-threatening and fatal bleeding

In second study of 7243 patients with UA/NSTEMI with no revascularization, prasugrel and ASA no better than clopidogrel and ASA in preventing risk of CV death, MI or stroke(Roe, et al 2012).

**Adverse effects:**

Contraindicated in pts with history of stroke or TIA
Not for patients ≥75 unless high risk (diabetes)—increased bleeding in older patients

H. Ticagrelor (Brilinta®)

**Mechanism of action:** like the thienopyridines (ticlopidine, clopidogrel, prasugrel) it inhibits platelet aggregation by binding the platelet P2Y12 receptor and thereby preventing the conformational change in the IIb/IIIa receptor that is necessary for fibrinogen binding and subsequent platelet aggregation. Unlike the thienopyridines, it does this reversibly.

**Pharmacology:** Not a pro-drug - no issues with conversion to active drug
Increased risk of non-CABG bleeding
Do not use >100 mg ASA/d with it
Dose: 90 mg/d; loading dose (180 mg)
Effect reversible: 3-5 days for platelet recovery

Clinical Uses:

PLATO study: ticagrelor + ASA vs clopidogrel + ASA
(Wallentin, et al 2009)
Ticagrelor
Had better reduction in death
No increased risk of bleeding
But increased risk-non-CABG bleeding
Trend to higher rate hemorrhagic stroke

North America subgroup (2011b)
No difference
Felt due to higher dose of ASA in North America

I. Comparing thienopyridines with ticagrelor

- Thienopyridines (clopidogrel, prasugrel)
  - Irreversibly bind P2Y12 receptor
  - Pro-drug variably metabolized to active drug
  - Prasugrel better converted to active drug than clopidogrel
  - Prasugrel more effective than clopidogrel
    - But more bleeding with prasugrel
- Ticagrelor
  - Reversibly binds P2Y12 receptor
  - Not a prodrug; no need for conversion
  - T + ASA better than C + ASA in CAD (not in USA pts)
  - Increased non-cardiac bleeding
  - Avoid >100 mg ASA/day

J. Vorapaxar (Zontivity®)

Mechanism of action: Thrombin is a potent activator of platelets via to PAR-1 receptor on platelets. Vorapaxar blocks PAR-1 and inhibits thrombin-induced platelet activation(2014b)

Pharmacology:
Oral
Tmax: 1-2 h
T1/2: 8 days
Dose: 2.08 mg tablet once a day
Addition to ASA and or clopidogrel not well studied
Metabolism: Strong CYP3A inhibitors increase and
inducers decrease vorapaxar exposure.
Platelet effect irreversible: given its long half-life effect may last weeks

Clinical Studies:

TRACER study (Tricoci, et al/2012) – 12,944 patients with acute coronary syndromes without ST changes standard of care with placebo or vorapaxar
Study stopped early due to excessive bleeding (inc ICH)
No advantage over placebo in composite endpoint of all of the following: death due to CV causes, MI, stroke, recurrent ischemia or urgent revascularization

TRA-2o (TIMI 50) (Morrow, et al/2012) – 26,449 pts with PAD, history of MI or stroke; placebo or vorapaxar
Stroke group stopped due to increased rate of ICH
Composite endpoint of CV death, stroke, or MI at 3 years was different: 9.3% with V and 10.5% with placebo
In group of 17,779 pts with prior MI, endpoint was 8.1% with vorapaxar vs 9.7% with placebo (Scirica, et al/2012)
3787 pts with PAD had no difference in composite endpoint but did have reduced acute limb ischemia (2.3% V; 3.9% P)

Adverse events: major bleeding risks
Moderate/severe bleeding: 4.2% with V; 2.5% placebo)
ICH: 1.0% V; 0.5% placebo)

K. Direct inhibitors of IIb/IIIa (mimic Glanzmann’s thrombasthenia)

1. ReoPro® - abciximab - monoclonal antibody vs IIb/IIIa.
5% of patients develop thrombocytopenia
immediate effect
effect lasts 24-48 h
increased risk of bleeding
coronary angioplasty, unstable angina

2. Integrilin® - eptifibatide - cyclic heptapeptide composed of lys-gly-asp-trp-pro-cys linked by a propionyl residue.
Blocks binding of fibrinogen to IIb/IIIa.
effect lasts 4 hours after infusion stopped
increased risk of bleeding
unstable angina, angioplasty

3. Aggrastat® - tirosiban - non-peptide blocker of IIb/IIIa -
C22H36N2O8S-HCl-H2O
effect lasts 4 hours
increased risk of bleeding
acute coronary syndromes, not elective angioplasty

L. Monitoring therapy

1. Tests of platelet function (Bleeding Time, Platelet Function Tests) may become abnormal but do not correlate with anti-thrombotic effect

2. Corollary is that anti-thrombotic effect can be shown epidemiologically in the absence of changes in tests

3. Better tests are needed!

M. Novel Anti-platelet agents

Monoclonal antibodies to GPIb - inactivate VWF receptor and prevent adhesion - mimic Bernard Soulier Syndrome

Inhibitors of prostaglandin synthesis or function

XI. COSTS OF PLATELET TESTS AND BLOOD PRODUCT TRANSFUSIONS (MGH 5/2017).

<table>
<thead>
<tr>
<th>Bone marrow biopsy/aspirate</th>
<th>$175</th>
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<tbody>
<tr>
<td>interpretation</td>
<td>175</td>
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Platelet tests - screening

| Bleeding time | $152 |
| Platelet count | 23 |
| PTT           | 33 |

Platelet tests - specific

| Platelet aggregation with ristocetin | $394 |
| VWF:RCo                                | 458 |

Blood product transfusions

| Type and cross-match | $171 |
| Plasma - one unit    | 317  |
| Platelets - one unit PC | 390 |
| Red blood cells - one unit | 637 |

XII. SELECTED REFERENCES


(2011b) Ticagrelor (Brilinta)--better than clopidogrel (Plavix)? *Medical Letter on Drugs and Therapeutics*, 53, 69-70.


(2014b) Vorapaxar (Zontivity) for prevention of thrombotic cardiovascular events. *Medical Letter on Drugs and Therapeutics*, 56, 85-86.


