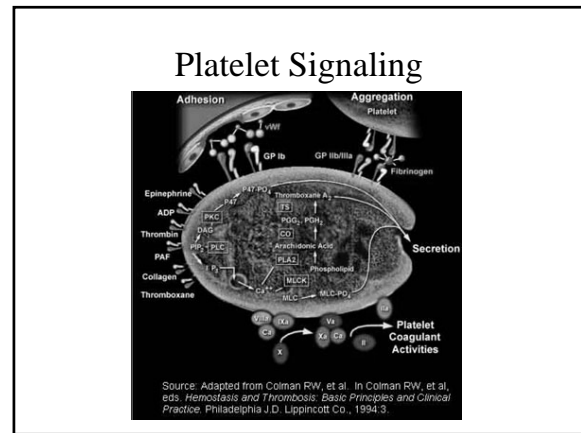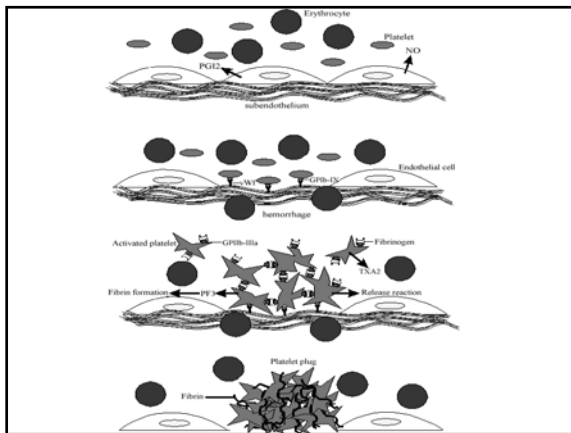# Qualitative and Quantitative Platelet Disorders

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Learning Objectives

- Understand the evaluation of thrombocytopenia and qualitative platelet abnormalities focusing on laboratory medicine.
- How are platelets measured using an automated cell counter? What artifacts can be seen with this automated counting?
- How is a bleeding time performed and is it a good test?
- Know how optical platelet aggregation, Accumetrics and PFA-100 can measure qualitative platelet abnormalities -- congenital platelet disorders, drug effect, von Willebrand Disease.

Platelet Signaling

Source: Adapted from Colman RW, et al. In Colman RW, et al, eds. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice.* Philadelphia J.D. Lippincott Co., 1994:3.

Platelet vs. Coagulation Bleeding

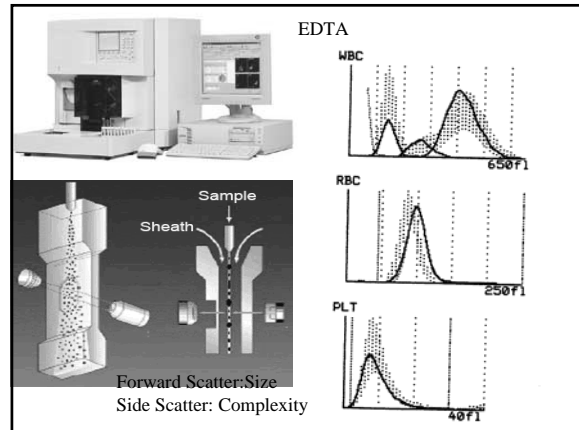| Findings	Coagulation	Platelet
Petechiae	Rare	Common
Hematomas and Hemarthroses	Common	Rare
Delayed Bleeding	Common	Rare
Bleeding cuts	Minimal	Persistent
Gender	Male	Women
Mucosal	Minimal	Typical

N.B. Some platelet disorders are associated with thrombosis (HIT, TTP)

Laboratory Tests

- Automated Cell Counter
  - Platelet Count
  - Mean Platelet Volume
  - Platelet Distribution
- Smear morphology
- Coagulation
  - PT, aPTT, Fib, TT
- Bleeding Time
- Aggregometry
  - Optical
  - Accumetrics
  - PFA-100
- vWD
  - Ristocetin Cofactor
 - vWf:Ag
  - FVIII:C
  - Multimers

Automated Cell Counter

- Platelets can be measured by the automated cell counter in two ways:
  - Impedence
    - Count: Cells a passed through a channel single file; the number of cells counted between 2 and 20 fL is the platelet count
    - Size: Area under the deflection curve is the size; the mean of all the areas if the mean platelet volume (MPV)
  - Optical
    - Polymethine fluorescent dye stains DNA/RNA and platelet membranes and granules
    - Laser counts the positively stained cells

EDTA

WBC

650fl

RBC

250fl

PLT

40fl

Sample

Sheath

Forward Scatter:Size
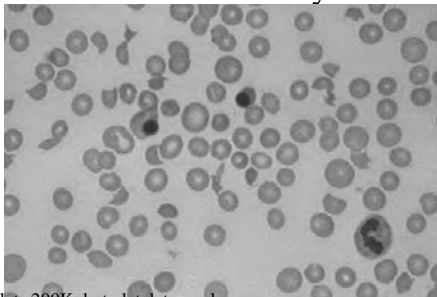Side Scatter: Complexity

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Real or Spurious?

22 year old student athlete undergoes a routine preoperative physical exam and laboratory studies prior to right knee arthroscopy; he has no significant past medical history; no bleeding or family bleeding history; he takes no medications; physical exam is unremarkable

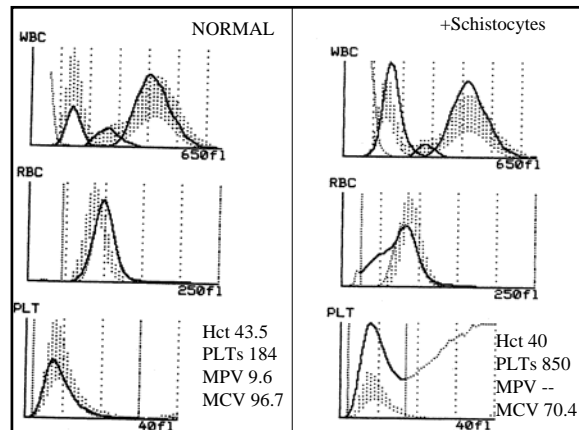WBC 5.0, Hct 45%, Plt 20K, smear next slide

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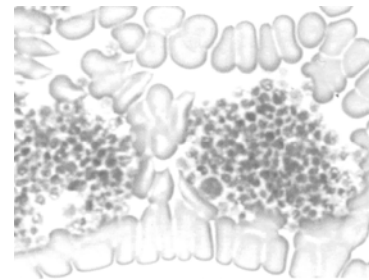
Pseudothrombocytopenia
Anti-coagulant dependent agglutinins associated with EDTA
Re-rerun with citrate or heparin tube

Real or Spurious?
Effect of Schistocytes

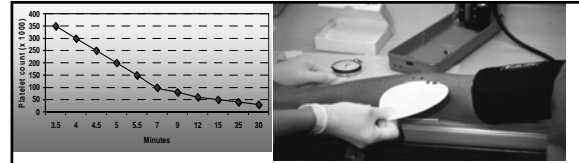Platelets 200K, but platelets rarely seen on smear

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NORMAL	+Schistocytes
WBC	WBC
650fl	650fl
RBC	RBC
250fl	250fl
PLT	PLT

NORMAL:
Hct 43.5
PLTs 184
MPV 9.6
MCV 96.7

+Schistocytes:
Hct 40
PLTs 850
MPV --
MCV 70.4

Real or Spurious?
Platelet Morphology
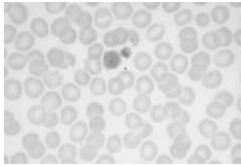
Large platelets may not fall into the platelet window

Bernard-Soulier

- To screen for inherited platelet dysfunction (e.g. vWD)
- Done under standardized conditions
  - 40 mmHg
  - Two small punctures on volar surface
  - Absorbed every 30 sec
  - Measured by time in minutes
  - Should not be done if plt<50K, anemia or uremia
- Mainly affected by platelet number and function, hematocrit
- There is no evidence that the bleeding time predicts bleeding
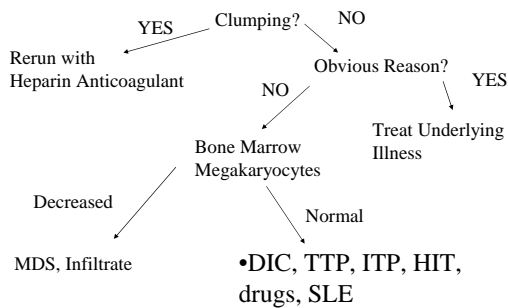- no correlation between bleeding time and visceral bleeding

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Bleeding Time Prolonged

- Congenital
- Drugs (e.g. antiplatelet drugs +/- ASA)
- Alcohol
- Uremia
- Hyperglobulinemias
- Fibrin/fibrinogen split products
- Thrombocythemia
- Cardiac Surgery

Evaluate as two groups

- Quantitative
  - Production, Destruction, Sequestration, Dilution

- OR

- Qualitative
  - Adhesion, Aggregation, Secretion, Medication

Thrombocytopenia on Automated Counter

YES   Clumping?   NO

Rerun with Heparin Anticoagulant

Obvious Reason?   YES

NO

Treat Underlying Illness

Bone Marrow Megakaryocytes

Decreased   Normal

MDS, Infiltrate

•DIC, TTP, ITP, HIT, drugs, SLE


Quantitative

- Production
  - Reduced Megakaryocytes
    - Infiltration (e.g. tumor)
    - Aplasia (e.g. chemicals)
    - Congenital (e.g. WAS)
  - Ineffective
    - Megaloblastic anemia, myelodysplasia, ETOH

- Destruction
  - Immune
    - Autoantibody e.g. ITP
    - Alloantibody
      - NAIT, HIT
  - Consumption
    - DIC
    - TTP
    - Mechanical
- Sequestration
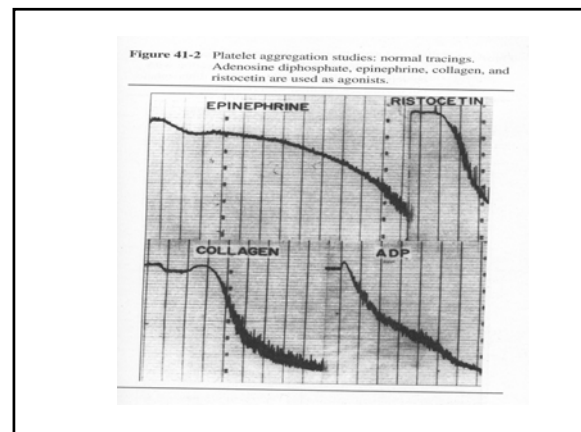- Hemodilution
- Real or Spurious?

Aggregometry

- Purpose: used to detect abnormalities in platelet function
- Principle: an aggregating agent is added to platelet rich plasma in a cuvette; as the platelets aggregate, the light transmission increases
- **Specimen: platelet rich plasma prepared from citrate whole blood with test completed within 3 hours of the collection**
- Procedure: soft spin to prepare platelet rich plasma prepared; hard spin to prepare platelet poor plasma (blank)

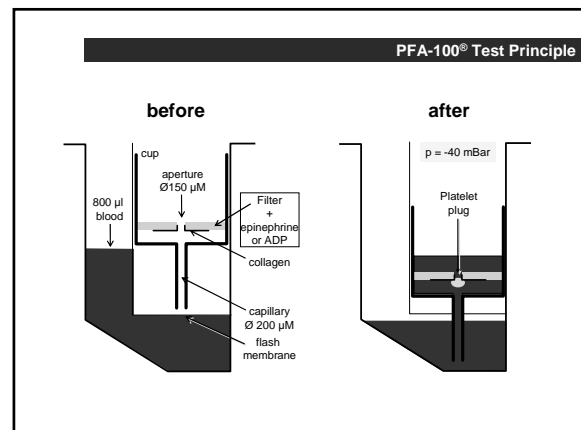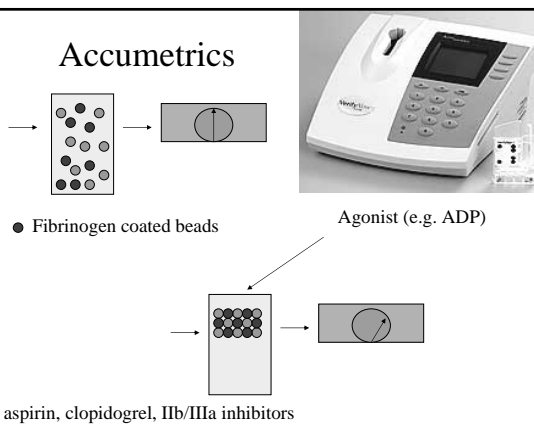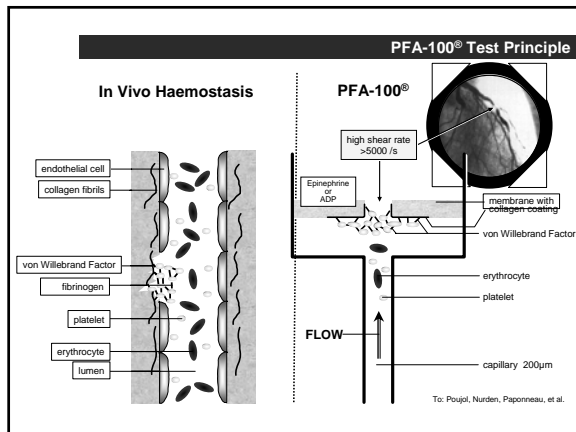Plt Poor Plasma Blank

Agonist (e.g. ADP)
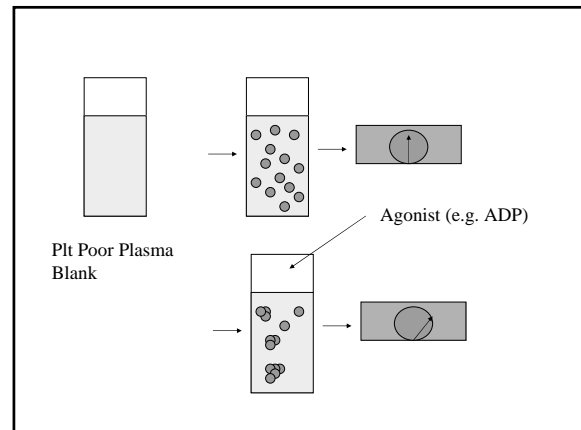
Interpretation

- Evaluate the slope of aggregation; both primary and secondary wave
- Evaluate the extent of aggregation
- Low dose ADP: two waves; high dose a single wave
- Epi biphasic in 80% of normal
- Collagen acts by releasing ADP so only a single wave
- Ristocetin antibiotic that makes vWf bind platelets and induces aggregation; normal tracing does not exclude vWD

Figure 41-2 Platelet aggregation studies: normal tracings. Adenosine diphosphate, epinephrine, collagen, and ristocetin are used as agonists.

EPINEPHRINE   RISTOCETIN

COLLAGEN   ADP

Accumetrics

● Fibrinogen coated beads

Agonist (e.g. ADP)

aspirin, clopidogrel, IIb/IIIa inhibitors

PFA-100® Test Principle

before   after

cup   p = -40 mBar

800 µl blood
aperture Ø150 µM
Filter + epinephrine or ADP
collagen
Platelet plug
capillary Ø 200 µM
flash membrane

PFA-100® Test Principle

In Vivo Haemostasis

PFA-100®

high shear rate >5000 /s

Epinephrine or ADP

membrane with collagen coating

von Willebrand Factor

endothelial cell
collagen fibrils
von Willebrand Factor
fibrinogen
platelet
erythrocyte
lumen

erythrocyte
platelet

FLOW

capillary 200μm

To: Poujol, Nurden, Paponneau, et al.

PFA-100

- Preoperative evaluation
- Menorrhagia evaluation
- Patient aspirin compliance or resistance or other drug induce platelet dysfunction
- Screening/evaluation of patients with suspected inherited or acquired platelet disorders
- Screening/evaluating von Willebrand disease (vWD)
- Evaluation of the bleeding patient
- Monitoring DDAVP treatment in patients with Type I vWD

Qualitative

- Inherited
  - Bernard-Soulier (Adhesion)
  - Glanzmann's (Aggregation)
  - Storage pool disease (Secretion)
    - ChediakHigashi, Wiscott-Aldrich, Hermansky-Pudlak, Gray Platelet Syndrome
- Acquired
  - Drugs (e.g. ASA, clopidogrel, IIb/IIIa inhibitors)
  - Uremia, Post-bypass
 - Primary marrow disorders; MDS, Dysproteinemias

Bernard-Soulier

- Rare inherited bleeding disorder
- Lack of GPIb which is necessary for the formation of the hemostatic plug by binding to subendothelial von Willebrand factor
- Aggregation with ADP, Epi and collagen; absent ristocetin

Glanzmann's Thrombasthenia

- Rare Condition
- Inherited absence of GPIIb/IIIa (AR)
- Severe Bleeding manifestations
- GPIIb/IIIa a key platelet glycoprotein required for aggregation
- Absence of aggregation with ADP, Epi, Collagen
- Normal ristocetin

Hermansky-Pudlak

- 21 month old male with bruisability and bleeding
- albino features
- Oculomotor nystagmus
- Delayed development
- tyrosinase-positive oculocutaneous albinism (Ty-pos OCA), bleeding diathesis, and systemic complications associated to ceroid-lipofuscin–like lysosomal storage disease.

Thrombocytopathies

- Common
- Abnormality in the release reaction
- Storage Pool Disease (no ADP in granules)
- Release defect (defects in mechanism of release)
- Resembles same pattern as aspirin
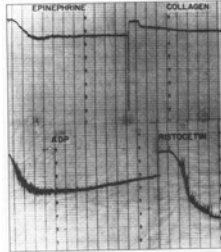
von Willebrand's Disease

- Inherited bleeding disorders
- Absent or decreased levels of vWf or lack of large and medium sized multimers
- Work up includes vWf:Ag level, FVIII:C activity, Ristocetin Cofactor Activity, Platelet Aggregation studies
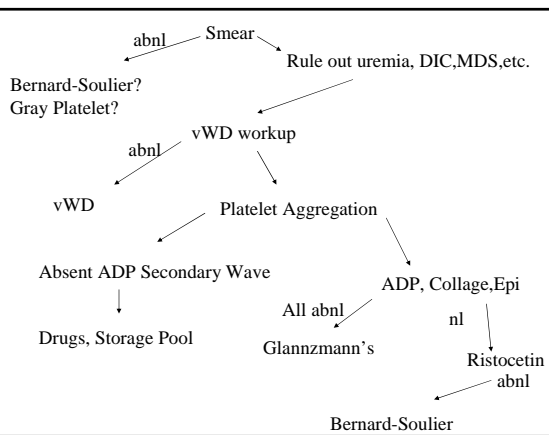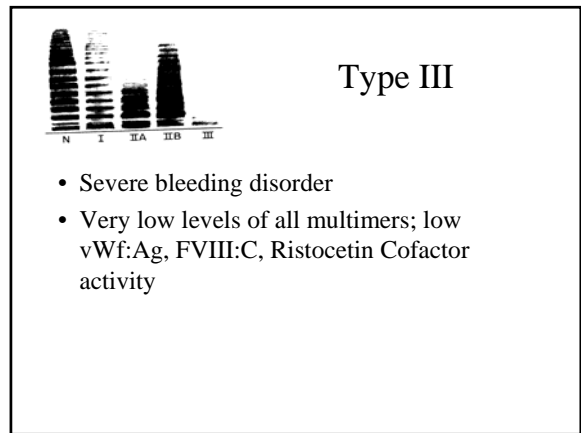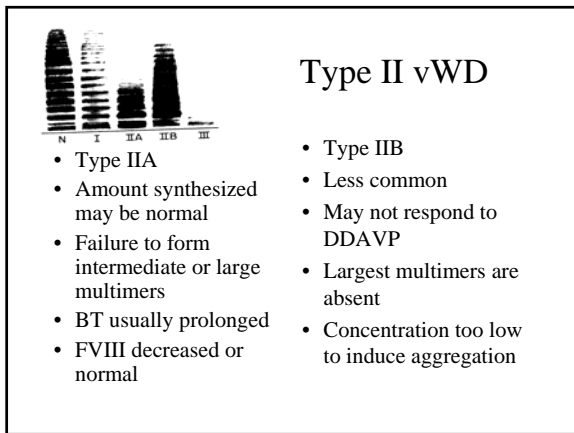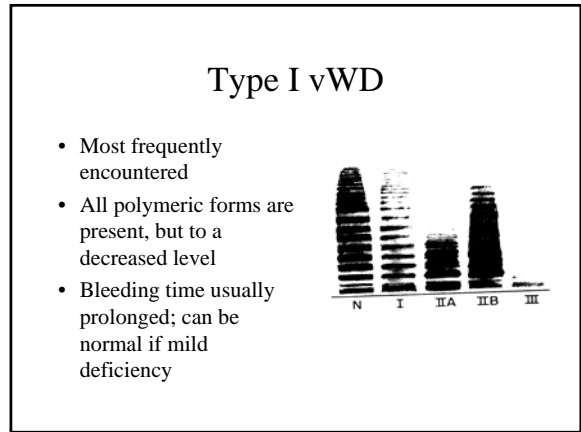

Case

- 33 year old woman with menorrhagia
- History of epistaxis since childhood
- Cousin with similar problems
- Aspirin for headaches; no other meds
- PT, PTT, TT, Platelets normal count
- Blood smear platelet morphology normal

Differential Diagnosis

- Inherited
  – Bernard-Soulier
  – Glanzmann's
  – Storage Pool Defect
  – vWD
- Acquired
  – DIC, MDS, uremia, drugs, dysproteinemia

vWD Lab Workup

- Bleeding Time
- Ristocetin Cofactor (functional)
- Ristocetin Aggregation
- vWf Ag (quantitative)
- Factor VIII:C
- Multimeric Analysis

vWD

Type I vWD

- Most frequently encountered
- All polymeric forms are present, but to a decreased level
- Bleeding time usually prolonged; can be normal if mild deficiency

Type II vWD

- Type IIA
- Amount synthesized may be normal
- Failure to form intermediate or large multimers
- BT usually prolonged
- FVIII decreased or normal

- Type IIB
- Less common
- May not respond to DDAVP
- Largest multimers are absent
- Concentration too low to induce aggregation

Type III

- Severe bleeding disorder
- Very low levels of all multimers; low vWf:Ag, FVIII:C, Ristocetin Cofactor activity

Test	IA	IIA	IIB	III
BT	V	V	V	V
FVIII	D	D or N	D or N	D
vWAg	D	N or D	N or D	D
Rist Cof	D	D	D or N	D
Rist Aggr	D or N	D	I	D
Multimer	N	A	A	A