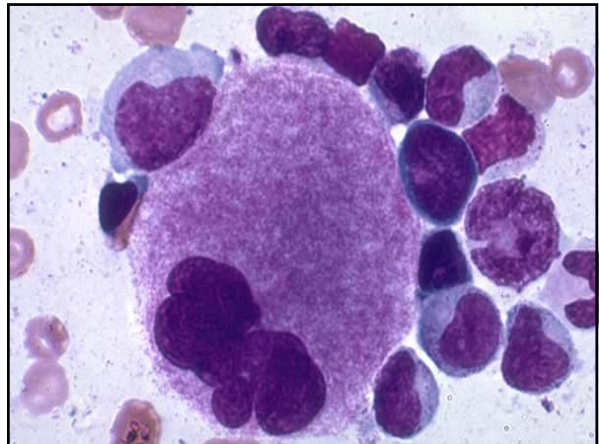


# The Evolving Role of Reticulated Platelets

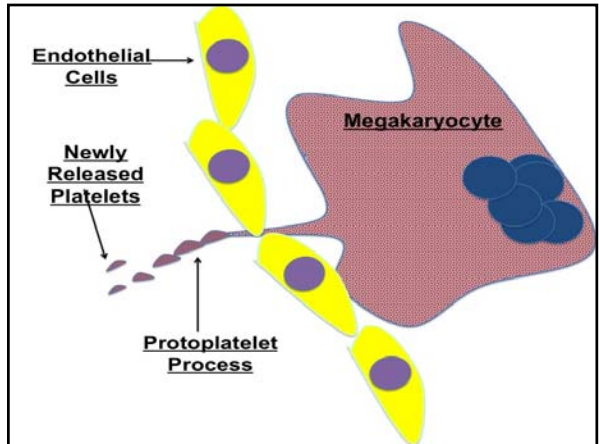
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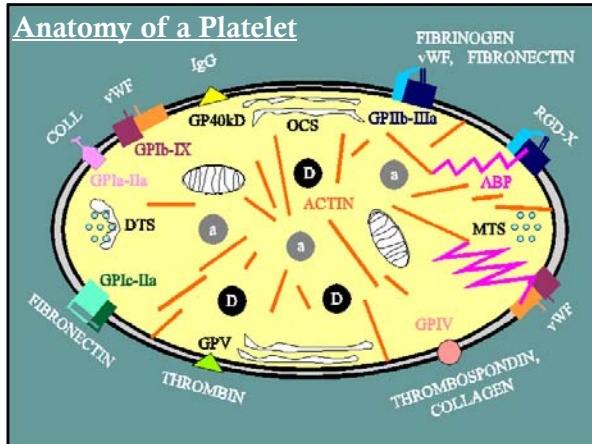


- ## Session Objectives
- Recall the process involved in megakaryopoiesis and thrombocytopoiesis
  - Explain the various methods involved in determining reticulated platelet concentrations
  - Discuss the clinical significance of reticulated platelets

- ## Platelets
- Thrombocytopoiesis
    - The formation of megakaryocytic cell fragments
    - Stimulated by IL-6 and IL-11
    - The megakaryocyte forms a proplatelet process that pieces between lining endothelial cells

- ## Platelets
- Megakaryocytopoiesis
    - Occurs within bone marrow
    - Stimulated by thrombopoietin (TPO)





### Determining Etiology

- **First step:** Physician performs history and physical to ID:
  - family history
  - recent exposures to viruses/drugs
  - Organomegaly
  - Bleeding locations

### Thrombocytopenia

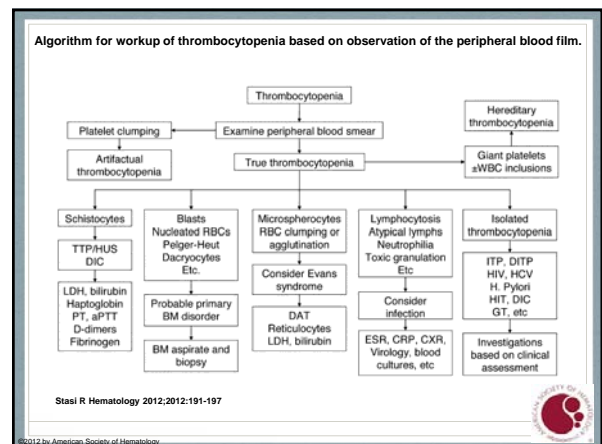
- Platelet count below the 2.5<sup>th</sup> lower percentile of normal
- Lower limit = 150,000/uL (NHANES III)
- Spontaneous bleeding usually does not occur until counts <20,000/uL
- Three major etiologies:
  1. Impaired or Decreased Production
  2. Increased Destruction
  3. Abnormalities of Distribution

### Determining Etiology

- **Second Step:** Laboratory Examination
  - Review of a peripheral blood smear is still the most important component!
  - Follow-up testing frequently required
  - BM aspirate examination may be required to clarify etiology

### Thrombocytopenia

Impaired Production	Increased Destruction	Distribution Abnorm.
Bernard-Soulier Syndrome	Immune	Inc. Splenic Sequestration
Fanconi Anemia	• ITP	Hypothermia
Wiskott-Aldrich Syndrome	• HIT	Loss of Platelets
May-Hegglin Anomaly	• Drug-Induced	Massive Blood Transfusion
Megaloblastic Anemia	Non-Immune	
Viral-Induced	• TTP	
Neonatal	• DIC	
	• HUS	



## Example: Suspected ITP

- CBC with Retic
- Peripheral Blood Film
- Immunoglobulin levels
- Bone Marrow Exam (for older patients)
- DAT
- Tests Inf. Agents (ex. HIV, HCV, H. pylori)

## Moving Forward

- Based upon the current landscape we need several things:
  1. Better accuracy at lower platelet counts
    - Segal et al (2012) study showed that overestimation still occurs
  2. Increased efficiency in providing clinically useful data for diagnosis

## Issues with Platelet Counts

- Automated cell counters produce reliable counts except in thrombocytopenia
  - Well that's helpful....
- Microscopic platelet estimates help confirm results from cell counters
  - But that takes time from an already busy staff...
- Platelet estimates can be accomplished in various manners
  - Quick literature search revealed 5 methods

## Moving Forward

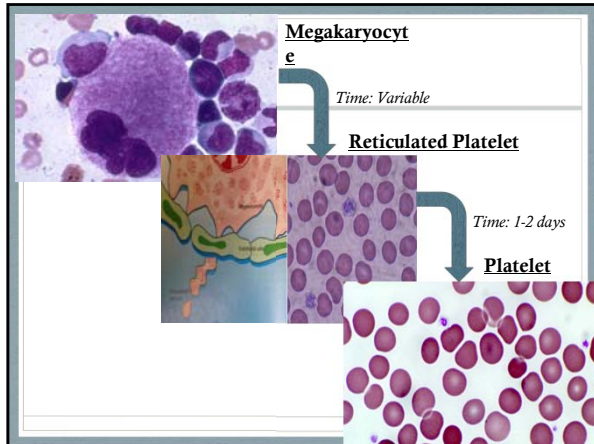
- Increasing accuracy of lower platelet counts needs further work
  - Optical and electrical impedance methods are still inadequate
- Increased efficiency in data production
  - RETICULATED PLATELETS?!?!?

## Issues with Platelet Counts

- Three problems with current state of platelet counts:
  1. Analytical reliability of automated counts during thrombocytopenia
  2. Turn-around-time for platelet estimates to confirm the counts
  3. Further follow-up testing still required

## Reticulated Platelets

- First characterized in 1967
- Newly released platelets contain residual RNA
  - Analogous to reticulocytes

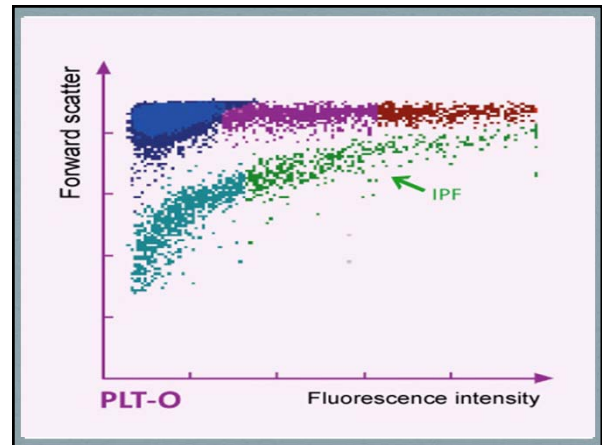


## Reticulated Platelets

- Methods
  - Flow Cytometry – Newer Methods
    - Sysmex’s Immature Platelet Fraction (IPF) was first to market
      - Uses proprietary mix of polymethine and oxazine
      - Pass sample through laser to obtain forward scatter (size) and fluorescence (RNA content)
        - Gating discriminates IPF
        - AUTOMATED!

## Reticulated Platelets

- Methods:
  - New Methylene Blue Stain
    - First method used for detection
    - Same concept as reticulocyte
    - Would you want to do this??



## Reticulated Platelets

- Methods
  - Flow Cytometry – Older Methods
    - Used thiazole orange to stain the nucleic acid content of platelets
      - Couple with CD41 or CD61
    - The fluorescent stain also stains some contents of dense granules
      - Falsely elevated counts
    - Expensive and labor-intensive

## Reticulated Platelets

- Methods
  - Flow Cytometry – Newer Methods
    - Abbott Cell-Dyn Sapphire was second to market with its reticulated platelet (rPT)
      - Uses proprietary CD4K530 dye to stain RNA of the platelets
      - Uses laser to detect size and fluorescence
      - Dependent upon presence of RBC
      - AUTOMATED!

## What Now?

- Reticulated Platelets can now be incorporated into routine practice
- Does the parameter give clinically useful information??

## Thrombocytopenia

Impaired Production	Increased Destruction	Distribution Abnorm.
Bernard-Soulier Syndrome	Immune	Inc. Splenic Sequestration
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Wiskott-Aldrich Syndrome	• HIT	Loss of Platelets
May-Hegglin Anomaly	• Drug-Induced	Massive Blood Transfusion
Megaloblastic Anemia	Non-Immune	
Viral-Induced	• TTP	
Neonatal	• DIC	
Chemotherapy-Induced hypoplasia	• HUS	

## Clinical Utility

- Initial Assessment of Thrombocytopenia
  - Clinician most likely concerned with cause being of hyper destructive or hypoplastic etiologies (to start)
  - Kurata et al (2001) found that relative reticulated platelet counts were excellent at diagnosing hyper destructive disorders
  - Measuring TPO and relative reticulated platelets simultaneously was found to be helpful in discriminating the cause of thrombocytopenia

## Clinical Utility

- Viral-Induced Thrombocytopenia
  - Hepatitis C can lead thrombocytopenia
    - Three proposed mechanisms:
      - TPO Suppression/Marrow Suppression
      - Autoimmune Destruction
      - Increased Sequestration

So What?

Who Cares?

What about specific platelets disorders?

## Clinical Utility

- Viral-Induced Thrombocytopenia
  - Hepatitis C
    - Zucker et al showed that IPF was better at distinguishing cause of thrombocytopenia
      - Increased %IPF correlated to autoimmune destruction of platelets
      - Decreased %IPF correlated to bone marrow suppression
    - Note: TPO levels were not associated to thrombocytopenia in this population

### Clinical Utility

- Neonatal Thrombocytopenia
  - Develops due to fetal exposure to:
    - CMV
    - HIV
    - Hepatitis
    - Toxoplasma
    - Rubella
    - Certain Drugs (ex chlorothiazide)
  - Exposure inhibits megakaryocytes and precursors from undergoing effective thrombocytopoiesis

### Clinical Utility

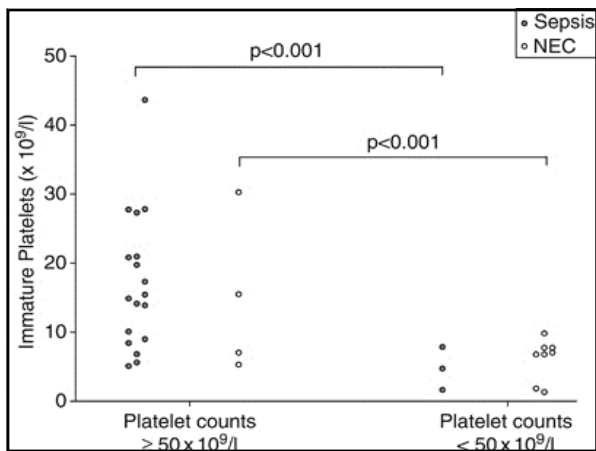
- Immune-Mediated Thrombocytopenic Purpura
  - Decrease in number of circulating platelets due to immune destruction of the platelets
  - Causes increase in thrombocytopoiesis to compensate (has no effect)

### Clinical Utility

- Neonatal Thrombocytopenia
  - Cremer et al. noted that this condition is frequently seen in NICU's
    - Increases risk of adverse outcomes and increases frequency of blood collection
  - %IPF < 8% increased risk of platelets dropping  $\geq 50,000/uL$  within one day
    - Relative Risk = 4.7
  - %IPF > 8% rarely indicated risk of worsening thrombocytopenia
    - 5 out of 99

### Clinical Utility

- Immune-Mediated Thrombocytopenic Purpura
  - Psaila et al found that patients with ITP had higher %IPF than controls
    - Indicative of increased thrombocytopoiesis
  - Treatment caused increase in absolute IPF but not in %IPF
    - Agrees with previous research by Barsam et al.



### Clinical Utility

- Post-Chemotherapy Platelet Recovery
  - Use of chemotherapy causes thrombocytopenia due to decreased number of megakaryocytes
  - Timing of platelet recovery varies widely from patient-to-patient and has made the use of prophylactic platelet transfusions difficult to manage



## Clinical Utility

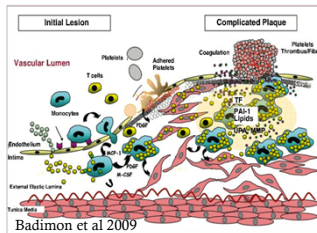
- Post-Chemotherapy Platelet Recovery
  - Wang et al (2002) used a calculated measure called the reticulated platelet maturation index (RP-MI)
  - Followed platelet counts and RP-MI from early in the nadir period to when recovery ( $\text{inc} > 20 \times 10^9/\text{L}$ ) began
  - RP-MI remained low in the earliest nadir period when recovery was not imminent
  - RP and RP-MI increased when recovery was imminent

## Conclusions

- Reticulated Platelets are platelets recently released into circulation
- Methods have existed since 1960's but new automation is making measurement easier
- The clinical utility has great potential and research continues into their role in assessing thrombocytopenia

## Clinical Utility

- Acute Coronary Syndrome
  - Platelets play a role in the process atherosclerosis



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- Cremer M et al. (2013): Low immature platelet fraction suggests decreased megakaryopoiesis in neonates with sepsis or necrotizing enterocolitis. *J Perinatol* 33(8): 622-626.
- Funck-Jensen K et al. (2012): Increased platelet aggregation and turnover in the acute phase of ST-elevation myocardial infarction. *Platelets* 24(7): 528-537.
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- Wang C, Smith BR, Ault KA, & Rinder HM. (2002): Reticulated platelets predict platelet count recovery following chemotherapy. *Transfusion* 42(3): 368-374.
- Zucker ML et al. (2012): Mechanism of thrombocytopenia in chronic hepatitis C as evaluated by the immature platelet fraction. *Int J Lab Hematol* 34: 525-532.

## Clinical Utility

- Acute Coronary Syndrome
  - Funck-Jensen et al (2012) found that increases in the immature platelet fraction indicated an elevated risk of acute myocardial infarction
  - Increased platelet turnover → Increased need