The Evolving Role of Reticulated Platelets

Kyle Riding, PhD, MLS(ASCP)CM
University of Central Florida

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)
Anatomy of a Platelet

Thrombocytopenia

- Platelet count below the 2.5th 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

Thrombocytopenia

<table>
<thead>
<tr>
<th>Impaired Production</th>
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Determining Etiology

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

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

Algorithm for workup of thrombocytopenia based on observation of the peripheral blood film.
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)

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

- 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

- 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

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?!!

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!

- 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

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

- 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??

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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

So What?

Who Cares?

What about specific platelets disorders?

Clinical Utility

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

Clinical Utility

• Viral-Induced Thrombocytopenia
  - 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 (e.g., 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

- 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 > 50,000/uL within one day
    - Relative Risk = 4.7
  - %IPF>8% rarely indicated risk of worsening thrombocytopenia
    - 5 out of 99

Clinical Utility

- 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 (inc >20 x 10^9/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

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

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

References