Hematology Tools to aid in the Diagnosis and Management of Thrombocytopenia

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OBJECTIVES

☑ Discuss the importance of providing accurate platelet counts and the clinical value of the Immature Platelet Fraction.

☑ Review the mechanisms of thrombocytopenia and disease states that are often associated with thrombocytopenia as a secondary side effect.

☑ Explain the advantages and disadvantages of the traditional lab tests for the diagnosis and treatment of thrombocytopenia.
THROMBOCYTOPENIA
DEFINITION OF THROMBOCYTOPENIA

- Traditional lower limit of normal is $150 \times 10^9/L$
- Platelet counts between $100 - 150 \times 10^9/L$ do not necessarily indicate disease if stable for 6 months
- IQMH Critical values can range from $20 - 70 \times 10^9/L$ for the lower limit and $750 - 1000 \times 10^9/L$ for the upper limit

IQMH: Institute for Quality Management in Healthcare
RELEVANCE OF THROMBOCYTOPENIA

- Aggravate surgical or traumatic bleeding
- Prevent the administration of effective treatment for several conditions
- A low platelet count is the only initial indication of an underlying disorder that poses greater risks than thrombocytopenia itself (e.g. HIV, infection)
- An important marker of disease activity (e.g. thrombotic microangiopathies)
IMPORTANCE OF ACCURATE PLATELET COUNT
IN THE NOT SO DISTANCE PAST
TODAY’S HEMATOLOGY ANALYZERS

Sysmex

Abbott

Beckman Coulter

Siemens

Horiba
IMPEDEANCE PLATELET COUNTING
OPTICAL PLATELET COUNTS

- Dual Light Scatter
- Reduces some interferences
  - Microcytic RBC
  - RBC Fragments
- Captures giant platelets
PLT-F CHANNEL

PLT performance

- Impedance platelet analysis (size) has limitations in the identification and discrimination of platelets from interfering particles with the same size.

Possible interferences

- RBC fragments counted as platelets: falsely high
- Microcytic RBCs counted as platelets: falsely high
- Large platelets counted as RBC: falsely low
ESTABLISHING THE MECHANISM OF THROMBOCYTOOPENIA
MECHANISMS OF THROMBOCYTOPENIA

- Decreased Production in the Bone Marrow
  - Aplastic anemia
  - Myelodysplastic syndromes
  - Chemotherapy-induced thrombocytopenia

- Increased Destruction / Consumption
  - DIC
  - Thrombotic microangiopathies
MECHANISMS OF THROMBOCYTOPENIA

- Platelet sequestration
  - Seen in congestive splenomegaly due to portal hypertension
  - Characterized by redistribution of platelets from the circulating pool to the splenic pool

- Hemodilution
  - Patients who have suffered a massive hemorrhage
  - Received colloids, crystalloids, and platelet-poor blood products
MECHANISMS OF THROMBOCYTOPENIA

- Multiple Mechanisms
  - Primary ITP
  - Hepatitis C virus infection
RELEVANCE OF THROMBOCYTOPENIA

Hospitalized patients
- Thrombocytopenia appears frequently in the background of a multisystem disorder

Outpatients
- Thrombocytopenia is often isolated and asymptomatic
- Diagnosis of the specific cause is usually straightforward
ESTABLISHING THE CAUSE OF THROMBOCYTOPENIA

Pregnancy

Possible consequences for the fetus
ESTABLISHING THE CAUSE OF THROMBOCYTOPENIA

Table 1. Clinical scenarios and most common causes of thrombocytopenia

<table>
<thead>
<tr>
<th>Outpatient</th>
<th>Multisystem illness/ICU</th>
<th>Cardiac patient</th>
<th>Pregnancy/postpartum</th>
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<tbody>
<tr>
<td><strong>ITP</strong></td>
<td>Infections</td>
<td>HIT</td>
<td>GT</td>
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<tr>
<td><strong>DITP</strong></td>
<td>TTP/HUS</td>
<td>Cardiac bypass</td>
<td>ITP</td>
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<tr>
<td><strong>Infections</strong></td>
<td>DITP</td>
<td>GPIIb/IIIa inhibitors</td>
<td>HELLP syndrome</td>
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<td>HIV</td>
<td>DIC</td>
<td>Other DITP</td>
<td>Preeclampsia</td>
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<td><em>Hepatitis C virus</em></td>
<td>Liver disease</td>
<td>Dilutional</td>
<td>Abruptio placenta</td>
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<tr>
<td><em>Helicobacter pylori</em></td>
<td>HIT</td>
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<td>TTP/HUS</td>
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<tr>
<td>CMV</td>
<td>MAS</td>
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<td>Other recent viral infections</td>
<td>BM disorders</td>
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<td><strong>Connective tissue disorders</strong></td>
<td>CIT</td>
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<td>Systemic lupus erythematosus</td>
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<td>Rheumatoid arthritis</td>
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<td>Antiphospholipid syndrome</td>
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<td>Vaccinations</td>
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<td>Myelodysplastic syndromes</td>
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<td>Congenital thrombocytopenia</td>
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<td>Common variable immunodeficiency</td>
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</table>

TTP/HUS indicates thrombotic thrombocytopenic purpura/hemolytic uremic syndrome; MAS, macrophage activation syndrome (including hemophagocytic syndrome); CIT, chemotherapy-induced thrombocytopenia; HELLP, hemolysis, elevated liver enzymes, and low platelets.
HISTORY & PHYSICAL EXAM –
ESTABLISHING A BASELINE

- New onset, chronic, or relapsing
- Family History
  - Autoimmune disorders, infections, or malignancies; pregnancy status in premenopausal woman; recent medications and vaccinations; recent travels (e.g., malaria, rickettsiosis, dengue fever); recent transfusions; recent organ transplantation; ingestion of alcohol and quinine-containing beverages; dietary habits; and risk factors for retroviral infections and viral hepatitis
HISTORY & PHYSICAL EXAM

- Bleeding history
  - Does not help in diagnosing the nature of the thrombocytopenia
  - Gives important clues about its duration and defines its clinical phenotype.
THE LABORATORY’S ROLE
THE LABORATORY’S ROLE

- Accurate Platelet Count
- Peripheral Smear Review
  - Still the most important diagnostic approach
- All 3 Blood Lineages should be examined
  - Thrombotic microangiopathy (Fragments)
  - Acute Leukemia (Blasts)
PLATELETS

- Platelet Clumping
  - EDTA Clumpers (1 in 1000 normal adults)
- Platelet size and granularity
- Hereditary disease
  - Macrothrombocytopenia
  - Gray platelet syndrome
  - Platelet destruction – large and normal size
  - Platelet production – normal
  - Myleodysplastic syndromes – variable size, frequently hypogranular
WBC

- Leukemia
- Lymphomas
- Neutrophilia, Lymphocytosis, Leukopenia
- Pelger Huet Anomaly

- Toxic Granulation associated with sepsis
RBC

- Schistocytes
  - Thrombotic microangiopathy (TTP/HUS)
  - DIC
- Microspherocytes
- Macrocytosis (with hypersegmented neuts)
- Dacrocytes (teardrops)

NRBC

- Hemolytic anemia
- Myelofibrosis
- Infiltration of the bone marrow
ALGORITHM BASED ON OBSERVATION OF THE PERIPHERAL BLOOD FILM

Thrombocytopenia

- Platelet clumping
  - Artifactual thrombocytopenia
    - Schistocytes
      - TTP/HUS DIC
        - LDH, bilirubin Haptoglobin PT, aPTT D-dimers Fibrinogen
    - Blasts Nucleated RBCs Pelger-Huet Dacrocytes Etc.
      - Probable primary BM disorder
        - BM aspirate and biopsy
    - Microspherocytes RBC clumping or agglutination
      - Consider Evans syndrome
        - DAT Reticulocytes LDH, bilirubin
    - Lymphocytosis Atypical lymphs Neutrophilia Toxic granulation Etc
      - Consider infection
        - ESR, CRP, CXR, Virology, blood cultures, etc
      - Investigations based on clinical assessment

- Examine peripheral blood smear
  - True thrombocytopenia
    - Hereditary thrombocytopenia
      - Giant platelets ±WBC inclusions
    - Isolated thrombocytopenia
      - ITP, DITP HIV, HCV H. Pylori HIT, DIC GT, etc
OTHER TESTING INFORMATION

- Liver function and Renal panels
- Clotting testing + D-dimer
- Lactate dehydrogenase

Bone Marrow Biopsy
There is no single hematologic or biochemical test that is conclusive for a given mechanism of thrombocytopenia.
PLATELET COUNTING TODAY

- Multiple Robust Methods
  - Impedance
  - Optical
  - Fluorescent
- Better Accuracy & Precision
- Confidence in low end counts?
New Platelet Parameters
MPV

- MPV – Mean Platelet Volume
  - Measures the average size of the platelet (similar to MCV for RBCs)
  - Calculated or derived parameter
  - May be used as an indicator of thrombopoiesis
MPV

Limitations

- Not all large platelets are young platelets
- MPV is derived or calculated from the platelet histogram

• What do you do if you don’t have a good histogram?
RETICULATED PLATELETS

- Newly released platelets, contain RNA and are **larger than** mature platelets
- They are the platelet analogue of red cell reticulocytes
- Are more reactive than mature platelets
- Reflect the rate of thrombopoiesis
- Platelet RNA content correlates with megakaryocytic activity
RETICULATED PLATELETS

- Distinction between causes of thrombocytopenia:
  - Marrow failure (low reticulated platelet count)
  - Increased marrow production secondary to peripheral platelet destruction/consumption (high reticulated platelet count)

- The RNA can be stained using supravital or fluorescent dyes
- Can potentially be quantitated by microscopy and flow cytometry
- Needs fluorescent label with nucleic acid affinity
REAGENT REACTION

RBC

PLT

IPF
IMMATURE PLATELET FRACTION - IPF

- % of total platelets that are immature
- Measures platelets newly released from the bone marrow
- Indicator of thrombopoiesis
PLT-F SCATTERGRAM - NORMAL PATTERN
DIFFERENTIATE PHYSIOLOGICAL MECHANISMS

Low PLT + Low IPF
Consistent with disorder of production

Low PLT+ High IPF
Destruction consistent with autoimmune or other destruction mechanism (ITP, TTP, DIC)

Normal
INTERFERENCE WITH ROUTINE IMPEDANCE COUNT

β-Thalassemia Major with numerous fragmented red cells

- PLT-I = 477*10^9/L
- PLT-O = 111*10^9/L
- PLT-CD61 = 152.2*10^9/L
- IPF% = 13.9%

- PLT-I = 514*10^9/L
- PLT-F = 140.8*10^9/L
- PLT-CD61 = 152.2*10^9/L
- IPF% = 12.9%
IMPROVED PERFORMANCE OF PLT-F

Acute Promyelocytic Leukemia / chemo: white blood cell fragments

PLT- O

WBC cytoplasm fragments

PLT- F

WBC cytoplasm fragments

PLT- I = 25*10^9/L
PLT- O = 181*10^9/L
PLT- CD61 = 24.2*10^9/L
IPF% = 41.2%
IPF# = 74.6*10^9/L

PLT- F = 28*10^9/L
PLT- F = 24.2*10^9/L
PLT- CD61 = 24.2*10^9/L
IPF% = 1.1%
IPF# = 0.3*10^9/L
BINDING SITES OF FLUOROCCELL PLT

Fluorocell PLT stains nucleic acid rich organelle
- Rough-surfaced endoplasmic reticulum (ribosomal RNA)
- Mitochondria (MtDNA)
BRIGGS, C.
BRITISH JOURNAL OF HEMATOLOGY

Ref Range IPF% 1.1 – 6.1% (7.0%)

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<td>Normals</td>
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<td>DIC</td>
<td>25</td>
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<td>Cancer</td>
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</table>
PLT-F SEPARATES PLATELETS FROM RBC FRAGMENT BY THE DIFFERENCES IN STAINING

- **Platelet**
  - CD41 (Green)
  - PLT-F (Red)
  - CD41/PLT-F (Yellow)

- **RBC Fragment**
  - CD41 (Green)
  - PLT-F (Red)
  - CD41/PLT-F (Yellow)

Transmitted light

- **PLT-F**
- **CD61/PLT-F**
POTENTIAL IPF APPLICATIONS

- TPO monitoring
- Bone Marrow recovery after Peripheral Blood Stem Cell transplant
- HIT
- Cardiology
Other Potential Uses of IPF
DRUG INDUCED THROMBOCYTOPENIA

- Usually by accelerating PLT destruction via drug-dependent anti-platelet antibodies
- Drug binds to platelet surface glycoproteins (GPIb-IX, GPIIb-IIIa) causing conformational change and exposing neoepitope that serves as target for antibodies
- Thrombocytopenia resolved after the suspected drug is discontinued
- Thrombocytopenia masked by the drug, appears after the suspected drug is discontinued
- Median for PLT count recovery after discontinuation of drug is 5-7 days
DRUG INDUCED THROMBOCYTOPENIA

The mostly likely culprits include:

- Quinine
- Quinidine
- Valproic acid
- Ranitidine
- Rifampin
- Trimethoprim-sulfamethoxazole
- GPIIbIIIa inhibitors
- Heparin

Others…
IN SUMMARY

Healthy bone marrows respond to decreased circulating platelets by releasing young platelets.

Thrombopoiesis is a dynamic process which has varying physiologic responses in different clinical conditions.

It is essential to determine the pathophysiologic cause of thrombocytopenia.

Young platelets can be recognized by both their size, and by their nucleic acid content.
IN SUMMARY

- The Lab’s role in aiding the physician in the diagnosis and treatment of thrombocytopenia is essential
  - Accurate and Precise Platelet count are critical
  - Peripheral Blood smear review is of upmost importance
  - The fully automated Immature Platelet Fraction is a helpful tool to the physician in the diagnosis and treatment of thrombocytopenia
QUESTIONS ?