Advances in the diagnosis and management of neonatal thrombocytopenia

Robert D. Christensen, MD

April 13, 2013
Outline

1) Reference ranges for platelet counts
2) Advances in understanding congenital hyporegenerative thrombocytopenias
3) Is it time for a new platelet transfusion paradigm?
4) Romiplostim and Eltrombopag
Outline

1) Reference ranges for platelet counts.

2) Advances in understanding congenital hyporegenerative thrombocytopenias

3) Is it time for a new platelet transfusion paradigm?

4) Romiplostim and Eltrombopag
Platelet “Reference Range” for Neonates

- >47,000 neonates from Intermountain Healthcare, Utah, USA
- All run on the same model of platelet counter (Beckman Coulter LH 750)
- Data excluded if diagnosis of SGA, PIH, sepsis.
Platelet Count At Birth

<table>
<thead>
<tr>
<th>Gestational Age (weeks)</th>
<th>5th %, mean, 95th %</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-24</td>
<td>100,000 - 300,000</td>
</tr>
<tr>
<td>25-27</td>
<td>300,000 - 400,000</td>
</tr>
<tr>
<td>28-30</td>
<td>400,000 - 500,000</td>
</tr>
<tr>
<td>31-33</td>
<td>500,000 - 600,000</td>
</tr>
<tr>
<td>34-36</td>
<td>600,000 - 700,000</td>
</tr>
<tr>
<td>37-39</td>
<td>700,000 - 800,000</td>
</tr>
<tr>
<td>40-42</td>
<td>800,000 - 900,000</td>
</tr>
</tbody>
</table>

Platelet Count At Birth

Feb 2009
Platelet Count, First 90 days After Birth

Age in Days

Platelet Count

Mean 5th %ile 95th %ile
Mean Platelet Volume

Platelet size varies.

The MPV is an electronic estimate of the average size (volume) of platelets in femtoliters (fL)
Sysmex® XE-Series Automated Hematology Analyzers
Since Changing to the Sysmex –

● Sometimes we don’t get a mean platelet volume (MPV). Why?

● What is the immature platelet fraction (IPF) reported on the Sysmex hematology analyzer?
• Platelet counts are identical between Sysmex and Beckman Coulter (impedance).

• If the Sysmex fails to register a ‘reliable’ platelet count it switches from the impedance (PLT-I mode) to an optical method (PLT-O mode) that does not measure MPV.

• Immature platelet fraction (IPF) can be measured on the Sysmex in the PLT-O mode.
- Forward scatter estimates platelet size
- Fluorescence intensity estimates platelet nucleic acid concentration
Immature Platelet Fraction

- Forward scatter estimates platelet size
- Fluorescence intensity estimates platelet nucleic acid concentration
- “Events” (dots) in the IPF “gate” (green) are “immature platelets”
• Forward scatter estimates platelet SIZE
• Fluorescence intensity estimates pl nucleic acid concentration

• “Events” (dots) in the IPF “gate” (green) are “immature platelets”

• Green dots divided by turquoise plus green dots = IPF (%).
● Forward scatter estimates platelet SIZE

● Fluorescence intensity estimates platelet nucleic acid concentration

● “Events” (dots) in the IPF “gate” (green) are “immature platelets”

● Green dots divided by turquoise plus green dots = IPF (%).

● IPF reference range for neonates, 2% to 5%

● Elevated IPF suggests active production (large, active) platelets
Outline

1) Reference ranges for platelets
2) Advances in understanding congenital hyporegenerative thrombocytopenias
3) Is it time for a new platelet transfusion paradigm?
4) Romiplostim and Eltrombopag
Most neonates with severe and prolonged thrombocytopenia have consumptive thrombocytopenia with a cause that is apparent (e.g. sepsis/NEC).

However some cases are congenital, persistent, and puzzling.
Congenital Hyporegenerative Thrombocytopenia

- Thrombocytopenia from birth, generally <15,000/µL
- Not Alloimmune or TORCH or SEPSIS
- Normal response to platelet transfusion
- Reduced or absent megakaryocytes in marrow, very high serum TPO level
Hyporegenerative Congenital Thrombocytopenia

- Small Platelets
- Normal Size Platelets
- Large Platelets
Hyporegenerative Congenital Thrombocytopenia

- Small Platelets: MPV < 5 fL
- Normal Size Platelets: MPV 8 to 12 fL
- Large Platelets: MPV > 15 fL
<table>
<thead>
<tr>
<th>Type of Platelets</th>
<th>MPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Platelets</td>
<td>MPV &lt;5 fL</td>
</tr>
<tr>
<td>Normal Size Cells</td>
<td>MPV 8</td>
</tr>
</tbody>
</table>

- Hyporegenerative Congenital Thrombocytopenia
- X-linked or Wiskott Aldrich syndrome
- Mutation in WAS at Xp11.23
Hyporegenerative Congenital Thrombocytopenia

Small Platelets
- MPV <5 fL

Normal Size Platelets
- MPV 8 to 12 fL

Large Platelets
- MPV >15 fL

Bernard Soulier [3q21.3, 17p13.2 or 22q11.21]

MYH9-related disorder[22q12.3]

von Willebrand disease type IIB  VWF [12p13.31]
Congenital Thrombocytopenia with Normal Size Platelets

Are Specific Orthopedic Forearm Anomalies Present?
YES or NO
Orthopedic anomalies

Inability to pronate/supinate forearm

- ATRUS = Amegakaryocytic Thrombocytopenia with RadioUlnar Synostosis

A mutation in the \textit{HOXA11} gene @ 7p15.2 (haploinsufficient) - Autosomal Dominant or \textit{de novo} mutation
Orthopedic anomalies

- TAR = Thrombocytopenia and Absent Radii

A microdeletion on 1q21.1 is necessary (haploinsufficient). This deletion plus a point mutation on the allele (RBM8A gene) – Autosomal Recessive
Orthopedic anomalies NO

CAMT = Congenital Amegakaryocytic Thrombocytopenia

A mutation in the TPO receptor gene *c-MPL* @ 1p34.2

Autosomal recessive, compound heterozygotes
Severe Hyporegenerative Congenital Thrombocytopenia and Normal Size Platelets

Forearm Defect?

Yes

ATRUS

TAR

No

SGA or other anomalies?

Yes

Jacobsen

RUNX1

PTPN11

No

CAMT
Severe Hyporegenerative Congenital Thrombocytopenia and Normal Size Platelets

- Forearm Defect?
  - Yes
  - No

- ATRUS
- TAR
- SGA or other anomalies?
  - No
  - CAMT

- RUNX1
- PTPN11

Symmetrical SGA, hyperteloric, developmental delay, **Microdeletion** 11q23 (Genomic Microarray)
Severe Hyporegenerative Congenital Thrombocytopenia and Normal Size Platelets

Forearm Defect?
  Yes
  ATRUS
  TAR

No
  SGA or other anomalies?
    Yes
    Jacobsen
    RUNX1
    PTPN11

    No
    CAMT

Symmetrical SGA, developmental delay, Microdeletion 21q22.11 (Genomic Microarray)
**RUNX1** on Chromosome 21 Encodes a Runt-Related Transcription Factor

- **Katzaki *et al.*** Siena. Microdeletions of 21q22.11 *Am J Med Genet* 2010

- **Christensen *et al.*** Utah. Microdeletions of 21q22.11 *J Perinatology* 2012

- **Click *et al.*** Seattle. Microdeletions of 21q22.11 *Am J Med Genet* 2011
Severe Hyporegenerative Congenital Thrombocytopenia and Normal Size Platelets

**Forearm Defect?**

- **Yes**
  - ATRUS
  - TAR

- **No**
  - SGA or other anomalies?
    - **Yes**
      - Jacobsen
    - **No**
      - RUNX1
      - PTPN11
      - CAMT

Noonan phenotype, SGA, developmental delay, Mutation in *PTPN11* 12q24.13 [c.218 C>T]
Severe Hyporegenerative Congenital Thrombocytopenia and Normal Size Platelets

Yes

No

ATRUS

TAR

SGA or other anomalies?

No

CAMT

RUNX1

PTPN11

Noonan phenotype, SGA, developmental delay, Mutation in PTPN11 12q24.13 [c.218 C>T]
Severe Hyporegenerative Congenital Thrombocytopenia and Normal Size Platelets

Forearm Defect?

Yes

No

ATRUS

TAR

SGA or other anomalies?

No

CAMT

Yes

RUNX1

PTPN11

Noonan phenotype, SGA, developmental delay, Mutation in PTPN11 12q24.13 [c.218 C>T]

Kratz C, Niemeyer CM et al. Germany. BLOOD 2005

Nunes et al. Portugal. BMJ Case Reports 2012

Christensen et al. USA. NEONATOLOGY 2013

Bambino Gesù Hospital, Rome
Severe Hyporegenerative Congenital Thrombocytopenia and Normal Size Platelets

Forearm Defect?

Yes: ATRUS, TAR

No: SGA or other anomalies?

Yes: Jacobsen, RUNX1, PTPN11

No: CAMT

Mutation in TPO receptor (MPL) @ 1p34.2
Outline

1) Reference ranges for platelets
2) Advances in understanding congenital hyporegenerative thrombocytopenias
3) Is it time for a new platelet transfusion paradigm?
4) Romiplostim and Eltrombopag
Platelet Transfusion in the 1950’s

Reduced mortality rate of patients with hemorrhage from thrombocytopenia due to acute leukemia.
Platelet Transfusion in Today's NICU

2% = Treatment of thrombocytopenic bleeding.
98% = Prophylaxis. Hope to prevent bleeding when platelets fall below arbitrary blood level.

In non-bleeding, thrombocytopenic neonates, what are the RISKS of prophylactic platelet transfusions? What are the BENEFITS?
Correlation: More NICU platelet transfusions = Higher mortality rate

Baer & Christensen 2009
Multiple platelet transfusions to NICU patients are, themselves, a risk factor for adverse outcome.
BENEFITS: Do prophylactic platelet transfusions prevent intraventricular hemorrhage?

1. Andrew - pl 50,000 vs. 150,000/µL. *J Pediatr* 1993…*No*

2. Del Vecchio - pl in the range 100,000 - 150,000/µL do not increase bleeding time *J Perinatol* 2008…*No*

3. Von Lindern - Platelet transfusion and IVH. Comparison of a “liberal” vs. “restrictive” guidelines. *Arch Dis Child* 2012…*No*

4. Sola (PAS 2012) - Relationship between platelet count and IVH in VLBW neonates?…*No*
Consider MORE than just the platelet count.
Adequacy of platelet plug formation depends on:
1) Platelet count
2) Platelet size
3) Platelet function
4) Platelet/endothelial interaction
Platelet plug formation may be predicted **better** by the PLATELET MASS than by the platelet count.

Platelet count X MPV = Platelet Mass
Is it more appropriate to base platelet transfusion decisions on platelet mass than on platelet count?

We speculated that doing so would:

1. Reduce the number of pl transfusions
2. Specifically reduce those pl transfusion that are not helpful
3. Reduce costs
4. Preserve a valuable resource
### 2007 Guidelines

**Transfuse if the:**

<table>
<thead>
<tr>
<th>Risk Group 1. (ECMO, bleeding or pre- or postoperative)</th>
<th>Platelets fall below 100,000/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Group 2. (Not bleeding and unstable)</td>
<td>Platelets fall below 50,000/µL</td>
</tr>
<tr>
<td>Risk Group 3. (Not bleeding and stable)</td>
<td>Platelets below 20,000/µL</td>
</tr>
<tr>
<td>Risk Group</td>
<td>2007 Guidelines</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------</td>
</tr>
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<td><strong>Risk Group 1.</strong> (ECMO, bleeding or pre- or postoperative)</td>
<td>Platelets fall below 100,000/µL</td>
</tr>
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<td>Platelets fall below 50,000/µL</td>
</tr>
<tr>
<td><strong>Risk Group 3.</strong> (Not bleeding and stable)</td>
<td>Platelets below 20,000/µL</td>
</tr>
<tr>
<td>Risk Group 2. (Not bleeding and unstable)</td>
<td>Platelets fall below 50,000/µL</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Risk Group 3. (Not bleeding and stable)</td>
<td>Platelets below 20,000/µL</td>
</tr>
</tbody>
</table>

Platelet count X MPV = Platelet Mass
“Mass” Guidelines Resulted in Fewer Platelet Transfusions with no Increase in Bleeding Problems

<table>
<thead>
<tr>
<th></th>
<th>% receiving one or more pl trans</th>
<th>IVH all grades</th>
<th>Pulmonary</th>
<th>Gastrointestinal</th>
<th>Cutaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007 Platelet Count</td>
<td>3.6%</td>
<td>5.0%</td>
<td>0.6%</td>
<td>2.0%</td>
<td>6.2%</td>
</tr>
<tr>
<td>2008 Platelet Mass</td>
<td>1.9%</td>
<td>4.5%</td>
<td>0.7%</td>
<td>1.4%</td>
<td>5.3%</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.002</td>
<td>0.36</td>
<td>0.52</td>
<td>0.27</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Recommend Abandoning Platelet COUNT-based Guidelines Adopting Platelet MASS-based Guidelines

<table>
<thead>
<tr>
<th>Risk Group 1. (ECMO, bleeding or pre- or postoperative)</th>
<th>Previous Guidelines</th>
<th>Present Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transfuse if the:</td>
<td>Transfuse if the:</td>
</tr>
<tr>
<td></td>
<td>Platelet count falls below 100,000/µL</td>
<td>Platelet mass falls below 800 fL/nL</td>
</tr>
<tr>
<td>Risk Group 2. (Not bleeding and unstable)</td>
<td>Platelet count falls below 50,000/µL</td>
<td>Platelet mass falls below 400 fL/nL</td>
</tr>
<tr>
<td>Risk Group 3. (Not bleeding and stable)</td>
<td>Platelet count falls below 20,000/µL</td>
<td>Platelet mass falls below 160 fL/nL</td>
</tr>
</tbody>
</table>
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Romiplostim

• Analog of thrombopoietin
• Developed by Amgen under the trade name Nplate
• FDA approved 2008 for long-term treatment for chronic ITP in adults who have not responded to other treatments.
• The wholesale cost of romiplostim if administered weekly (adults) is about $55,000 per year.
• IV or sub Q use only
Eltrombopag

- Small molecule agonist of the Thrombopoietin receptor
- Discovered as a result of research collaboration between GlaxoSmithKline and Ligand Pharmaceuticals.
- FDA approved in 2008 for adults with ITP refractory to other treatments
- Oral preparation only
Romiplostim and Eltrombopag have been used in very few neonates.

Those few had chronic, transfusion-dependent, hyporegenerative thrombocytopenias or severe liver disease (TPO is produced by hepatocytes).
PREDICTION: These medications will likely have a limited value in the majority of cases of neonatal thrombocytopenia, but will benefit neonatal patients with certain uncommon varieties.

1) Not rapid-acting (10 days)
2) Not effective for most TPO receptor mutations (CAMT)
3) May elevate platelet count to safe range with certain hyporegenerative thrombocytopenias (*MYH9, RUNX1*)
4) Likely to reduce pl transfusions for neonates with liver diseases
1) Reference ranges for platelet counts: gestational and post-natal age.

### First Platelet Count By Gestational Age

<table>
<thead>
<tr>
<th>Gestational Age</th>
<th>Platelet Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5th %ile</td>
</tr>
<tr>
<td>5 days</td>
<td>87900</td>
</tr>
<tr>
<td>10 days</td>
<td>90400</td>
</tr>
<tr>
<td>15 days</td>
<td>93400</td>
</tr>
</tbody>
</table>

### First Platelet Count By Age in Days

<table>
<thead>
<tr>
<th>Age in Days</th>
<th>Platelet Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean 5th %ile 95th %ile</td>
</tr>
<tr>
<td>0</td>
<td>111000</td>
</tr>
<tr>
<td>7</td>
<td>110850</td>
</tr>
<tr>
<td>14</td>
<td>110000</td>
</tr>
</tbody>
</table>

Feb 2009
Recap

2) Advances in understanding congenital hyporegenerative thrombocytopenias
Recap

3) Is it time for a new platelet transfusion paradigm?
Recap

4) Romiplostim and Eltrombopag
Evidence-based neonatology hematology = better outcomes for NICU patients
"Ordem e Progresso"

L’amour pour principe et l’ordre pour base; le progrès pour but (Love as a principle and order as the basis; progress as the goal)

Thanks for your kind attention!

Advances in the diagnosis and management of neonatal thrombocytopenia