The Story of the Platelet Clump

Wayne Hall
MTN Network Laboratory
Magee-Womens Research Institute
Pittsburgh, PA
Reason for Alarm?

Platelet Clumping was occurring in multiple African sites 1-2 times per week in the months September to November, 2010.
The Culprit!!
Outline:

1. Development of Platelets & their Function
2. Laboratory Evaluation of Platelets in Clinical Trials
3. Platelet Clumping
4. Platelet Histograms & Pictures
5. Resolution to Platelet Clumping in EDTA tubes
6. Is platelet clumping a reason for Alarm?
7. Conclusions
8. Questions
The Birth of a Platelet
in the bone marrow

Hematopoietic stem cell → Promegakaryocyte → Megakaryocyte → Released in blood stream → Platelet
What is this?

Megakaryocyte
What fact is incorrect about Platelets?

1. Cytoplasmic fragments from Megakaryocytes formed in the bone marrow
2. Does not contain a nucleus or any DNA (contains enzymes & mitochondria)
3. **One-Third** (not 1/8\(^{th}\)) of the total blood platelets can be found in the spleen
4. Life span of a platelet is 5-7 days
5. Normal Count ~ 140,000 to 440,000
6. 30,000 to 50,000 platelets released in blood daily
Primary Platelet Functions
also called Primary Hemostasis

- **Maintenance of normal Hemostasis**
  - the opposite of hemostasis is hemorrhage

- **Adhere to the injury site**
  - due to chemical signal released by smooth muscle damage
  - exposed collagen fibers attracts platelets

- **Clump together (aggregate) with other platelets**

- **Release compounds that stimulate further aggregation**
Secondary Hemostasis

- Platelets support the Coagulation Cascade
- Formation of fibrin strains that are woven through the loose platelet plug forming a fibrin net
The Platelet Plug
Laboratory Evaluation of Platelets in Clinical Trials

- To accurately assess the platelet concentration in the peripheral blood

- To distinguish low platelet counts that can potentially be harmful to participants.

- To Identify participants with a platelet anomaly that would defer them from the study.
In Voice, what is the Platelet count Exclusion Criteria?

A. Platelet count below 75,000/mm$^3$
B. Platelet count below 100,000/mm$^3$
C. Platelet count below 125,000/mm$^3$
D. Platelet count below 150,000/mm$^3$
In ASPIRE (MTN-020), what is the Platelet count Exclusion Criteria?

A. Platelet count below 75,000/mm\(^3\)
B. Platelet count below 100,000/mm\(^3\)
C. Platelet count below 125,000/mm\(^3\)
D. Platelet count below 150,000/mm\(^3\)
Exclusion Criteria for Clinical Trials

In VOICE (MTN-003) platelet count below 100,000/mm³

In ASPIRE (MTN 020) platelet count below 125,000/mm³

*Based on the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December, 2004 (Clarification dated August 2009)
Sticky Situations

Platelet clumping in EDTA tubes

– creates illusion of low platelet count (pseudo-thrombocytopenia)
– Possibly will effect WBC count
– Creates extra work to resolve the problem
– Not a good candidate for enrollment
Causes for Clumped Platelets

• Faulty Phlebotomy practice

• Cold Agglutinins

• EDTA-Dependent Auto antibodies

• Unknown
Faulty Phlebotomy Practice:

- Delay in transferring blood into EDTA tube from a syringe.
- Difficult phlebotomy or slow blood flow into tubes.
- Overfilling tubes.
- Insufficient tube mixing.

These practices are preventable with good phlebotomy technique!!

**SIDE NOTE ABOUT BLOOD DRAWS:**
Make sure that your phlebotomists ID each participant every time they are taken into a new room...even if they just performed testing with that participant in another area!!!
Cold Agglutinins

• An antibody that attaches to platelets at temperatures colder than 34°C.
• Can be triggered by cold weather, change of environment, poor circulation

  – Warm the EDTA tube for 15-20 minutes in a 37 degree water bath or heat block, quickly vortex, and run immediately.
EDTA-Dependent Auto-antibodies

• Rare condition that occurs when an IgG antibody forms in the presence of EDTA

• Antibody coats the platelets, the platelets can rosette around segmented neutrophils, bands, and sometimes around monocyte.

• Slide may show platelet satellites (satellitism)

• Redraw blood into a Sodium Citrate tube and run on analyzer. Beware to account for the dilution factor (usually multiply result by 1.1).
Unfortunately, not all platelet clumping issues can be resolved.

If no reason can be determined at screening, this is not a good candidate to enroll!!
How are Platelet Clumps found?

• Analyzer flag for platelet clumps or giant platelets.

• Histogram will indicate an abnormal distribution curve

• Abnormal RBC Parameters: Cold agglutinins to Platelets & RBCs may cause indices such as MCHC (generally greater than 37) to be affected.

• Slide Review
Cell Analyzer Flags for Platelet Clumps or Giant Platelets

• All Platelet Clump and Giant Platelet flags will require a slide review

• The slide review will have both a WBC & Platelet estimate performed.
Platelet size histogram:
PL = the lower size threshold for the measurement of platelets
PU = the upper size threshold for the measurement of platelets
PDW = Platelet Distribution Width
P-LCR = Percentage of Large Cell Ratio
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### Cellular Interference

### Suspect/Definitive Messages:

- NRBC
- Platelet Clumps

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**08 OCT 2010**

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### Lab Results

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<td>MO %</td>
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### Suspect/Definitive Messages

- Platelet Clumps
- Thrombocytopenia

### Verify Diff

- Cellular Interference

### Report Information

- **Last Modified:** 02/15/2008
- **By:** LabAdmin
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- RBC: 4.83 10^12/L
- HGB: 13.3 g/dL
- HCT: 0.399 L/L
- MCV: 82.6 fl
- MCH: 27.5 pg
- MCHC: 33.3 g/dL
- RDW: 13.3 %
- PLT: 148 R L 10^9/L
- MPV: 10.4 R fl

### Suspect/Definitive Messages:

- Giant Platelets
- Nodules
- Clumping present
- Slide 160
- Dots

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**Plt Histogram**

![Plt Histogram]

**Giant Platelet**

![Giant Platelet]

**Nodules Clumping present**

![Nodules Clumping present]
Slide reviews

Wrights Stain: Platelet clumps
Platelet Satellitism

Platelet Clumping due to antibody-mediated, EDTA-dependent phenomenon.

Note how the platelets do not satellite around the eosinophil & monocyte.
Giant Platelets

- Giant Platelets can cause some instruments to give a “clumped platelet error”.

- On a slide, a platelet larger than the size of the average red blood cell, assuming a normal MCV, is considered “giant”.

- Can affect the WBC count
Giant Platelets
Resolution to Platelet Clumping in EDTA tubes

Instrument Flag & Errors:
   Platelet Clumps, Giant Platelets, Rejected Platelet counts

1. Check for Clots (use 2 applicator sticks)
2. Vortex tube for 15-20 seconds and rerun
3. Pull a slide for a slide review
4. If clumping seen on slide, pre-warm tube, vortex, and rerun.
5. Redraw into a citrate (or ACD) and EDTA tube. Correct for dilution factor.
6. If a manual platelet count (by a cytometer) is not available, then estimate count and comment: Adequate, low, or high.
7. If the manual platelet count can be performed, then a finger stick is performed, and blood is directly introduced into a diluting fluid.
Is Platelet Clumping a reason for Alarm?

• At present, there does not appear to be an abnormally high number reported from sites.
• New platelet clumpers are not being enrolled
• Recurrent clumpers are being drawn in citrate tubes.
• Your labs are handling the situations well!
Do we know why this happened last year from Winter to Spring?

No

Conjecture:
- Tube Inequality
- Colder weather than normal
- Poor phlebotomy technique
Conclusions

- Not all platelet clumping issues will be resolved (In Pittsburgh, LA, & Hawaii, approximately 10-15% of patient clumping in tubes goes unresolved).
- Upon screening, do not accept participants that have platelet clumps (unless due to poor phlebotomy technique, and the sample would be redrawn).
- Once enrolled, if clumping can not be resolved by drawing a citrate tube, then estimate platelet count from slide and determine if number is adequate.