Clinical Applications: Donor and Cellular Therapy

Jay S. Raval, MD
University of North Carolina
Chapel Hill, NC
Conflicts of Interest

• None
Objectives

- Donor Collections of Red Blood Cells (RBCs), Plasma, Platelets (PLTs), and Granulocytes
  - Apheresis Products
  - Characteristics of Apheresis Products
  - Indications for Transfusion of Blood Products
  - Adverse events during apheresis donation
Objectives

• Donor Collections of Hematopoietic Progenitor Cells (HPCs)
  – Clinical Indications
  – Autologous versus Allogeneic
  – Mobilization of Cells Into Peripheral Blood
  – Apheresis Product Collections
  – Adverse Events During Apheresis Donation
  – Products after Apheresis Collection
Donor Apheresis Products

- Apheresis Red Blood Cells
  - Leukoreduced (LR) vs. Not LR
- Apheresis Plasma
- Apheresis Platelets
  - LR vs. Not LR
- Apheresis Granulocytes
Donor Apheresis Products

• Multiple components can be collected from a single apheresis donor session
  – PLT + Plasma
  – RBC + Plasma
  – RBC + PLT
  – RBC + PLT + Plasma
  – 2-RBC
Apheresis Red Blood Cells

- Average collection of \( \geq 60 \) g hemoglobin/unit
  - Or 180 mL RBCs/unit
- 95% of units sampled >50 g hemoglobin
  - Or 150 mL RBCs/unit
Apheresis Red Blood Cells
Leukocytes Reduced

• Average collection of $\geq 51$ g hemoglobin/unit
  – Or 153 mL RBCs/unit
• $<5 \times 10^6$ leukocytes/unit
• $>95\%$ of units sampled $>42.5$ g hemoglobin/unit
  – Or 128 mL RBCs/unit
When To Transfuse RBCs?

• To improve **oxygen-carrying capacity**

• Simple transfusion
  – Anemia: <7 g/dl or <10 g/dl and symptomatic
  – Large volume blood losses

• Apheresis
  – Red Blood Cell Exchange
  – RBC prime when ECV >10-15% of patient’s TBV
  – As first part of TPE if HCT <21-25%
When Not To Transfuse RBCs?

- Down-trending Hgb/HCT
- Improve sense of well-being
- Promote wound healing
- ________________ said so
Apheresis Plasma

• Fresh frozen plasma (FFP)
  – Volume with citrate anticoagulation often between 250-350 ml
  – Placed at ≤-18 C within 8 hours of collection
    • Good for 1 year at this temperature
  – Content: “normal” levels of all coagulation factors
    • 1 IU/ml
  – Expiration: 24 hours after thawing
  – ABO compatibility required, cross-matching not required

AABB Standards for Blood Banks and Transfusion Services, 29th Ed.
Apheresis Plasma

• Thawed Plasma
  – After 24 hours, thawed FFP can be relabeled as Thawed Plasma and stored (aged) for 4 days @ 1-6°C
  – All coagulation factors maintained, except Factor V/VIII, which declines to 70% of normal
    ▪ Hemostatic level = 30-35%
  – Eliminates product wastage
  – Improves turnaround time
  – Interchangeable with FFP
Why Transfuse Plasma?

• In a bleeding patient or patient undergoing an invasive procedure who has a **SIGNIFICANT** coagulopathy.

• When is a coagulopathy **significant**?
How do we evaluate clotting?

**Intrinsic pathway**
- XII
- XI
- IX
- VIII

**Extrinsic pathway**
- VIIa
- PT/INR

**aPTTT**

**Thrombin**

**Fibrinogen**

**Fibrin Clot**
Why do *in vitro* coagulation tests fail to predict bleeding?

Mildly abnormal test results are associated with hemostatically adequate coagulation factor concentration.

When to Transfuse Plasma?

• Simple Transfusion
  – To control or prevent bleeding in patients with a documented clotting factor deficiency
    • Active bleeding/invasive procedure with: PT or aPTT prolonged > 4 secs (INR > 1.6)
    • Massive transfusion & bleeding w/ lab proven coagulopathy
    • Congenital deficiency of II, V, X, XI, XII
  – Emergency reversal of Warfarin
    • Subcut or IV Vit K reversal ⇒ 6-8 hrs
    • Now many use Prothrombin Complex Concentrates (PCCs)
When to Transfuse Plasma?

• Apheresis
  – Use of plasma gives back something important
    • TTP – ADAMTS13, haptoglobin
    • CAPS – anticoagulation factors
  – Use of plasma maintains hemostasis
    • Recent biopsy or operation
    • Planned biopsy or operation within 24-48 hours
    • Active bleeding – DAH in ANCA/GBM Disease
When to NOT Transfuse Plasma?

• Volume expansion
• Protein supplementation
• Does NOT reverse heparin, LMWH, or fondaparinux

• Prophylactic use with massive blood transfusion
• Prophylactic use after cardiopulmonary bypass
• Factor VII, VIII, IX, or ATIII deficiency
Apheresis Platelets

- ≥90% of units sampled have
  - ≥3.0 x 10^{11} PLT
  - pH ≥ 6.2 at time of issue/end of storage

- For Apheresis Platelets Leukocytes Reduced
  - Same PLT count and pH thresholds
  - ≥95% of units sampled have < 5 x 10^6 leukocytes
Why Transfuse Platelets?

- To control or prevent bleeding due to deficiencies of platelet number or function
  - Significant bleeding in a patient with thrombocytopenia
  - Planned invasive procedure in a patient with thrombocytopenia
  - Risk of spontaneous bleeding (e.g. CNS, lung) due to severe thrombocytopenia
  - Bleeding or invasive procedure and platelet dysfunction
Why Transfuse Platelets?

- Simple transfusion
  - <10,000/μl
  - <20,000/μl and febrile/unstable
  - <50,000/μl and
    - Actively bleeding
    - Invasive procedure
    - Trauma
  - <100,000/μl and neurosurgical procedures
  - Any platelet count if dysfunctional and bleeding
Why Transfuse Platelets?

• Apheresis
  – Safely place a patient on procedure
    • Anticoagulation puts patient at risk of bleeding
      – I.e, heparin in HPC collection or ECP
      – Targets usually >50,000/μl
    • Placement and/or removal of central venous access
      – Often for prevention of bleeding
      – Targets usually >50,000/μl
  • Procedure will decrease patient’s platelet count
    – Autologous HPC,A collection
When to NOT Transfuse Platelets?

- PLT CNT >100,000/μL w/o platelet dysfunction
- ITP, TTP, HIT unless bleeding is life-threatening
- Prophylactic use with massive blood transfusion
- Prophylactic use following cardiac bypass
This Slide’s For Free!

• Why should you specifically use apheresis platelets?
  – To control or prevent bleeding in patients refractory to random platelets (HLA-matched or HPA-matched platelets)
  – To reduce donor exposures in patients receiving a limited number of transfusions (compared to whole blood derived platelets)

• Otherwise, same as for random platelets
Apheresis Granulocytes

• Must contain $\geq 1.0 \times 10^{10}$ granulocytes/unit in $\geq 75\%$ units sampled

• To achieve this, additional agents must be used
  – Sedimenting agents
    • Hydroxyethyl starch induces RBC aggregation
    • Facilities must define and control the maximum amount of this used in a given time period due to side effects
      – Intravascular volume expansion
      – Severe pruritus $\rightarrow$ anaphylactoid reactions
      – Coagulopathy (via decreases of Factor VIII and vWF)
Apheresis Granulocytes

– Corticosteroids
  • Can increase circulating granulocytes by 200%
  • 60 mg prednisone p.o. vs. 8 mg dexamethasone p.o. administered 24 hours prior to collection
  • Donors with DM, cataracts, HTN, PUD - contraindicated

– Granulocyte colony stimulating factor (G-CSF)
  • Can increase circulating granulocytes by 500-1000%
  • 5-10 μg/kg administered 8-12 hours prior to collection

• With these strategies, potentially $1 \times 10^{11}$ or more granulocytes per collection can occur

Strauss RG et al, Vox Sang 2011.
When To Transfuse Granulocytes?

• Condition that has **reversible marrow suppression AND has bacterial (or fungal) infection that is not responding to standard antimicrobial therapy**

• Acceptable conditions
  – Post-HPC transplant, post-infectious aplastic anemia, marrow shock syndromes

• Unacceptable conditions
  – Metastatic disease to marrow, hypocellular conditions of marrow
Special Qualifications for Apheresis Donors

• Same as for whole blood...with a few exceptions

• Frequent plasmapheresis donor (source plasma)
  – More frequently than once every 4 weeks
    • At least 2 days apart, and ≤ 2x in any 7 days
  – Every donation tested for: HIV 1/2, Hepatitis B/C
  – Testing every 4 months for
    • Syphilis (Non-reactive)
    • Total plasma/serum protein (>6.0 g/dL)
    • SPEP or quantitative immunodiffusion assay (WNL)
    • Annual physical examination
Special Qualifications for Apheresis Donors

- Infrequent plasmapheresis donor (source plasma)
  - Less frequently than once every 4 weeks
  - Treated like a new donor every time
- Maximum plasma losses
  - 110-175 lbs → 12L/12 months
  - >175 lbs → 14.4L/12 months
- If no other components donated, malarial risk factors not a cause for deferral
Special Qualifications for Apheresis Donors

• 1-unit erythrocytapheresis (“single RBC unit”)
  – 8 week donation interval

• 2-unit erythrocytapheresis (“double RBC unit”)
  – 16 week donation interval
  – Hgb/Hct criteria dependent on
    • Donor gender
    • Specific apheresis instrument
    • Hct ≥ 40%
  – Donation will not drop Hgb/Hct <10 g/dL
Special Qualifications for Apheresis Donors

• Plateletpheresis donors may donate
  – Single, double, or triple product
  – 2x/week (at least 2 days apart)
  – 24x/rolling 12-month period
• Start PLT ≥150,000/µL and End PLT ≥100,000/µL
  – Can be performed before starting collection, or
  – Use average of previous pre-procedure counts, or
  – Default count
  • If qualifying PLT count not determined prior, split product
    should not be collected from 1st-time donors
Special Qualifications for Apheresis Donors

• No aspirin or piroxicam within 48 hours
• No clopidogrel or ticlopidine within 14 days
• May donate if whole blood donated within 8 weeks AND extracorporeal volume of instrument <100 mL

• Plasma volume collected/collection
  – ≤500 mL if 110-175 lbs, or
  – ≤600 mL if >175 lbs, or
  – Per instrument specifications
Special Qualifications for Apheresis Donors

- Apheresis Granulocytes donation
  - Can usually donate no more than 8-12 times/12 month period
  - Not evidenced based restriction, and exceptions may be made
  - Donors often recruited from plateletpheresis donor pool or patient’s relatives/social network
  - ABO/D status is respected, but can be mismatched if medical need outweighs risk

Strauss RG et al, Vox Sang 2011.
## RBC Losses and Deferral

<table>
<thead>
<tr>
<th>1&lt;sup&gt;st&lt;/sup&gt; RBC Loss</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; RBC Loss Within 8 week period</th>
<th>Deferral</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 300 mL</td>
<td>-</td>
<td>16 weeks</td>
</tr>
<tr>
<td>&lt; 200 mL</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; + 2&lt;sup&gt;nd&lt;/sup&gt; losses total ≥ 300 mL</td>
<td>16 weeks</td>
</tr>
<tr>
<td>200-299 mL</td>
<td>-</td>
<td>8 weeks</td>
</tr>
<tr>
<td>&lt; 200 mL</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; + 2&lt;sup&gt;nd&lt;/sup&gt; losses total = 200-299 mL</td>
<td>8 weeks</td>
</tr>
<tr>
<td>&lt; 200 mL</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; + 2&lt;sup&gt;nd&lt;/sup&gt; losses total &lt; 200 mL</td>
<td>None</td>
</tr>
</tbody>
</table>

Guidance for Industry and FDA Review Staff: Collection of Platelets by Automated Methods, ucm073382
Apheresis-Associated Adverse Events

• Many of these are similar to those seen with whole blood donations
• Some differences exist
  – Instrumentation used for collection
  – Frequency
## Incidence of tabulated adverse events

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Donor category</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First-time (2,295)</td>
<td>Repeat (17,303)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
<td>Number</td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td><strong>Citrate effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea and/or vomiting</td>
<td>20</td>
<td>0.87</td>
<td>46</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Tetany or seizure</td>
<td>2</td>
<td>0.09</td>
<td>7</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>0</td>
<td>0.01</td>
<td>2</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><strong>Vasovagal effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pallor and/or diaphoresis</td>
<td>43</td>
<td>1.87</td>
<td>55</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Nausea and/or vomiting</td>
<td>20</td>
<td>0.87</td>
<td>23</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Syncope and/or seizure</td>
<td>9</td>
<td>0.39</td>
<td>7</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Pulse &lt;50</td>
<td>0</td>
<td>0.02</td>
<td>3</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Pulse &gt;120</td>
<td>0</td>
<td>0.02</td>
<td>3</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure &lt;80</td>
<td>0</td>
<td>0.02</td>
<td>3</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td><strong>Venipuncture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe pain</td>
<td>10</td>
<td>0.44</td>
<td>21</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Nerve damage</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Palpable hematomata</td>
<td>48</td>
<td>2.09</td>
<td>176</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Donor category</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First-time (2,295)</td>
<td>Repeat (17,303)</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
<td>Number</td>
</tr>
<tr>
<td>Other severe events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chills and/or rigors</td>
<td>7</td>
<td>0.31</td>
<td>2</td>
</tr>
<tr>
<td>Arrhythmia (not citrate induced)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cardiopulmonary events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Circulatory collapse</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malfunctions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemolysis</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Air embolus</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clot or leak</td>
<td>5</td>
<td>0.22</td>
<td>10</td>
</tr>
<tr>
<td>Inability to return blood</td>
<td>6</td>
<td>0.26</td>
<td>26</td>
</tr>
</tbody>
</table>

## Nonvenipuncture adverse-effect rates for different donation procedures

<table>
<thead>
<tr>
<th>Donation procedure</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet (n = 17,584^*)</td>
<td>185</td>
<td>1.05</td>
</tr>
<tr>
<td>Granulocyte (n = 594^*)</td>
<td>4</td>
<td>0.67</td>
</tr>
<tr>
<td>Plasma (n = 1,359^*)</td>
<td>5</td>
<td>0.37</td>
</tr>
</tbody>
</table>

---

Side Effects Within 4 Weeks After Granulocyte Donation

Officially licensed collegiate product of the University of North Carolina at Chapel Hill
Apheresis HPC Collections

• Q: Why collection HPCs?
Apheresis HPC Collections

• A1: Patient has a hematopoietic disease that requires direct ablation of their native HPC population, and will need reconstitution of this cell population

• A2: Patient has a non-hematopoietic disease that requires medical treatment which will result in the ablation of their native HPC population, and will need reconstitution of this cell population
Conditions That May Require HPC Collection

- Hematologic Cancers
  - Multiple Myeloma, Lymphomas, Leukemias
- Marrow failure states
  - Myelodysplasia, PNH, aplastic anemia
- Pediatric malignancies
  - Rhabdomyosarcoma, medulloblastoma, Ewing Sarcoma, neuroblastoma, Wilms tumor
- Non-neoplastic disorders
  - Inborn errors of metabolism/immunodeficiency, thalassemia, SCD, autoimmune diseases
Autologous versus Allogeneic

- Autologous HPC donors
  - Donating to oneself
  - Perfect HLA Match
  - No risk of graft versus host disease (GVHD)
  - No benefit of graft versus tumor effect
  - No risk of infectious disease transmission
    - However, bacterial contamination is still a risk
  - Control of your own destiny
Autologous versus Allogeneic

• Allogeneic HPC donors
  – Healthy individual donating to a patient
    • Related versus unrelated
  – Not an HLA-identical Match
  – Risk of graft versus host disease (GVHD)
  – Benefit of graft versus tumor effect
  – Risk of infectious disease transmission
    • Bacterial contamination is still a risk
  – Not in control of your own destiny
Mobilization

- The process by which HPCs located in the marrow are released into the peripheral blood

- Many ways to do this
  - Granulocyte Colony Stimulating Factor (G-CSF)
    - 5-20 ug/kg/day, maximum yields after ~5 days
    - Can increase peripheral blood CD34 counts 2-10X
  - Chemotherapy (for autologous donors only)
    - Can increase peripheral blood CD34 counts 50-100X

Mobilization

– Plerixafor

• CXCR4 antagonist, can increase peripheral blood CD34 counts 2X+

• CONS: Expense, Scheduling of Administration (peak CD34 counts 10-14 hours after injection...perhaps even longer!)

Harvey et al. BBMT 2013
Product Collections

• Vascular Access
  – Most autologous donors will require placement of central venous catheters for HPC, Apheresis collection
  – 10% of male allogeneic donors and 20% of female allogeneic donors will require central venous catheters

Favre G et al. BMT 2003.
Product collections

• Anticoagulants of choice:
  – ACD-A
  – Heparin
  – Combination of both
Anticoagulation

Heparin

- Systemic anticoagulation
  - Extracorporeal circuit
  - Patient
- Higher flow rates
- Disadvantages:
  - Bleeding
  - Platelet/cell clumping
  - HIT

Citrate

- Extracorporeal anticoagulation
  - Donor/patient NOT anticoagulated
- Lower flow rates
- Lower pH → prevents plt clumping
- Rapidly metabolized (by liver)
- Disadvantages:
  - Hypocalcaemia
  - Metabolic alkalosis
  - Hypotension
Volume to Process

• Conventional volume HPC,A collections
  – 2-3 TBV (10-15 L in adults)
• Large volume HPC,A collections
  – 4-6 TBV (20-30+ L in adults)
• The more TBV that is processed...
  – The more CD34 cells collected from pt
  – The greater the platelet/granulocyte loss from pt
  – The greater the risk of anticoagulation/procedure complications
Adverse Events

• Complications from mobilization
  – G-CSF: Bone Pain, Headache, Fatigue, Myalgias, Nausea/Vomiting, Fever/Chills, splenic rupture (rare), acute lung injury (rare)
  – Plerixafor: Nausea, diarrhea, local skin reaction, headache, paresthesias
Adverse Events

• Complications of Central Venous Catheters
  – During placement: arterial puncture, pneumothorax, hemothorax, hematoma, pain
  – After placement: thrombosis, infection

• Complications of the apheresis procedure
  – Hypocalcemia/citrate-related toxicities, cytopenias, vasovagal reactions (diaphoresis, pallor, hypotension, bradycardia), hypovolemic hypotension, syncope

HPC,A Products: After Apheresis

• Where does the product go?
  – HPC Laboratory

• Product is:
  – Characterized (CD34 counts, TNC, viability)
  – Manipulated
    • RBC depletion, plasma depletion
  – Fresh infusion versus Cryopreservation
  – In general, minimum CD34 transplant dose required is $2 \times 10^6$/kg
Thank you for your attention!