Apheresis: Basic Principles, Practical Considerations and Clinical Applications

Joseph Schwartz, MD
Director, Transfusion Medicine
Columbia Univ. Medical Center
New York Presbyterian Hospital

Anand Padmanabhan, MD PhD
Assoc Med Director/Asst Prof
BloodCenter of Wisconsin
Medical College of Wisconsin

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Objectives (Part 1)

• Mechanism of Action
• Definitions
• Technology (ies)
• Use
• Practical Considerations
• Math
• Clinical applications – HPC Collection
Objectives (Part 2)

- Clinical applications: System/Disease Specific Indications
- ASFA Fact Sheet
Apheresis

• Derives from Greek, “to carry away”

• A technique in which whole blood is taken and separated extracorporeally, separating the portion desired from the remaining blood.

• This allows the desired portion (e.g., plasma) to be removed and the reminder returned.
Apheresis - Mechanism of Action

• Large-bore intravenous catheter connected to a spinning centrifuge bowl

• Whole blood is drawn from donor/patient into the centrifuge bowl

• The more dense elements, namely the RBC, settle to the bottom with less dense elements such as WBC and platelets overlying the RBC layer and finally, plasma at the very top.
Apheresis: Principles of Separation

Platelets (1040)

Lymphocytes (1050-1061)

Monocytes (1065 - 1069)

Granulocyte (1087 - 1092)

RBC
Separate blood components is based on density with removal of the desired component.
Principals of Apheresis
Apheresis - Mechanism of Action

TPE Channel

Plasma Out

RBC, WBC, and Platelet Return

Whole Blood In
Definitions

• **Plasmapheresis**: plasma is separated, removed (i.e. less than 15% of total plasma volume) without the use of replacement solution.

• **Plasma exchange (TPE)**: plasma is separated, removed and replaced with a replacement solution such as colloid (e.g. albumin and/or plasma) or combination of crystalloid/colloid.

Szczepiorkowski et al, Clinical Applications of Therapeutic Apheresis, J Clin Apheresis 2007, 22, 104-105.
Plasmapheresis/TPE: Fluid Dynamics

- INTRACELLULAR
  - K
  - 28 L

- EXTRACELLULAR
  - Na
  - 14 L

- INTERSTITIAL
  - 10 L

- INTRAVASCULAR
  - 4 L
Plasma Exchange: Mathematical Models

Modified from: Weinstein, Apheresis: Principles and Practice - AABB press
Figure 15-1. A model for the interaction between intravascular and extravascular compartments and the effects of plasma exchange. A soluble substance enters the body through the intravascular compartment at the synthetic rate SR, and is catabolically removed from the body from the intravascular compartment at its fractional catabolic rate (FCR). Movement from the intravascular to the extravascular compartment takes place primarily by diffusion while a smaller component of transmembrane flow occurs by other mechanisms. Soluble substances return from the extravascular compartment back to the intravascular compartment mainly through the lymphatic system, although a small amount of back-diffusion takes place. Plasma exchange directly removes soluble substances only from the intravascular compartment. SR, FCR, and intracompartment movement of each solute are balanced and thus in a steady state so proceed much more slowly than the actual removal of plasma from the intravascular compartment by plasma exchange. Therefore, for the purpose of therapeutic plasma exchange, the intravascular compartment is considered to be an isolated system that can be depleted of its soluble contents by the exchange of plasma for a replacement fluid.
Technology

• Automated centrifugal cell separators allow large of blood to be processed in a short period of time

• Discontinuous flow: Haemonetics MSC plus, V50, V30

• Continuous flow: Cobe spectra, CS 3000, Fresnius AS 104, Spectra optia
Use of Apheresis

• **Donor** - facilitate collection of a blood component from an allogeneic donor: Platelets, Granulocytes, source plasma, HPC collection

• **Therapy** (therapeutic apheresis):
  *removing undesired substances like antibodies, lipids*
  *reducing excess WBC/Platelets*
  *automated exchange of sickled RBC*
  *HPC collection*
Use of Apheresis (cont.)

Therapeutic apheresis assures the immediate removal of abnormal substances from the circulation, which are either:

* present in plasma

* or tightly bound to plasma proteins
Abnormal Substances Removed From the Circulation by TPE

1) Paraproteins (Waldenstrom’s Macroglobulinemia)

2) Autoantibodies (Myasthenia Gravis, Goodpasture’s syn.)

3) Lipids (LDL in familial hypercholesterolemia; phynatic acid in refsum’s disease)

4) Toxins or drugs (that are bound to albumin)

5) Circulating immune complexes (CIC)

6) Soluble mediators of inflammatory response (activated complement component, vasoactive substances)
Apheresis Procedural Elements (+ Practical Considerations):

• Venous access
• Replacement fluid
• Normal/abnormal constituents removed
• Anticoagulation
• Patient history and medications
• Frequency and number of procedures
• Complications
Apheresis Procedural Elements (+ Practical Considerations):

- Venous access
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- Frequency and number of procedures
- Complications
Venous Access

*Apheresis require large bore venous catheters to sustain the flow rates required (50-100 ml/min)

**Type of catheters**: 17 gauge therumo butterflies

- double lumen dialysis catheters 10-13.5 fr (Shiley, Quinton, Vascath, Permacath)

- Avoid “standard” Hickman or triple-lumen designs: flow rates are inadequate

**Location**: Peripheral: antecubital fossa

central: femoral/subclavian/jugular

arteriovenous shunt/fistula

**Number of lines**: intermittent flow devices (draw and return via the same line): single line

- continuous flow devices: separate lines
Venous Access (cont.)

- Planned/occasional procedure - peripheral line and removal after the procedure
- Few days/ bed rest- femoral line (risk of infection/thrombosis)
- Multiple procedures for a long period of time - neck central vein or arteriovenous shunt/fistula
- Do not forget:
  * Dressing change
  * Flush
Apheresis Procedural Elements (+ Practical Considerations):

• Venous access
• Replacement fluid
• Normal/abnormal Constituents Removed
• Anticoagulation
• Patient History and Medications
• Extracorporeal Volume
• Frequency and number of procedures
Replacement Fluid

- Must be FDA approved to use w/blood products [get mixed w/rbc before the return phase]

- Replacement solutions:
  - *Crystalloids – normal saline 0.9%
  - *Colloids – 5% albumin; plasma
Replacement Fluid

*The primary function of the replacement fluid is to maintain intravascular volume

**additional features:

- Restoration of important plasma proteins
- Maintenance of colloid osmotic pressure
- Maintenance of electrolyte balance
# Replacement Fluids

| TTP/HUS                  | FFP  
|-------------------------|------|
|                         | Cryodepleted FFP  
|                         | Mixtures: Albumin / FFP  
|                         | Albumin / FFP  
| Neurological            | 5% Human Albumin  
| GBS, MG, Stiff-man CIDP | Albumin / Saline (70% / 30%)  
| Renal                   | 5% Human Albumin  
| (RPGN, FSGS)            | Albumin / Saline (70% / 30%)  
| Post Transplant         | 5% Human Albumin  
|                         | Albumin / Saline (70% / 30%)  
|                         | Consider adding FFP at the end if post op  

Patients with hepatic failure, coagulopathy, pre-op or post-op use FFP or finish with FFP.
# Comparison of Replacement Fluids

<table>
<thead>
<tr>
<th>Replacement Fluid</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystalloid</td>
<td>Low cost</td>
<td>Hypo-oncotic</td>
</tr>
<tr>
<td></td>
<td>Hypoallergenic</td>
<td>No coagulation factors</td>
</tr>
<tr>
<td></td>
<td>No infectious risk</td>
<td>No immunoglobulins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-3 volumes required</td>
</tr>
<tr>
<td>Albumin</td>
<td>Iso-oncotic</td>
<td>Higher cost</td>
</tr>
<tr>
<td></td>
<td>No infectious risk</td>
<td>No coagulation factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No immunoglobulins</td>
</tr>
<tr>
<td>Plasma</td>
<td>Immunoglobulins</td>
<td>Infectious risk</td>
</tr>
<tr>
<td></td>
<td>Coagulation factors</td>
<td>Citrate</td>
</tr>
<tr>
<td></td>
<td>Iso-oncotic</td>
<td>Allergic reactions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABO compatibility</td>
</tr>
</tbody>
</table>
Replacement Fluid and Balance

3 choices of fluid balance (FB):

1) 100% FB – isovolemic – volume replaced = volume removed

2) <100% FB – hypovolemic (“dry”) - volume replaced < volume removed

3) >100% FB – hypervolemic (“wet”) - volume replaced > volume removed
Apheresis Procedural Elements (+ Practical Considerations):

• Venous access
• Replacement fluid
• Normal/abnormal constituents removed
• Anticoagulation
• Patient history and medications
• Frequency and number of procedures
• Complications
Normal/abnormal Constituents Removed

TPE:

• One volume exchange removes about 63%-65% of most plasma constituents

• A single two-volume exchange removes about 86% of plasma constituents

⇒ Increasing the volume beyond 1-1.5 volumes has very little impact on removal of plasma constituents
Volume of Patient Plasma Exchanged (PEX)

1 pv = 63% ↓, 2 vol = 86% ↓, 3 vol = 95%

Volume of Patient Plasma Exchanged (PEX)

- Little advantage beyond 1.0-1.5 volumes
  
  1 pv= 63% ↓, 2 pv=86% ↓, 3 pv=95%

- Removal of IgG and IgM by plasma exchange:

<table>
<thead>
<tr>
<th>measure</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>intravascular amount</td>
<td>45%</td>
<td>76%</td>
</tr>
<tr>
<td>“total body” removal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 PEX vol.</td>
<td>28%</td>
<td>48%</td>
</tr>
<tr>
<td>1.5 PEX vol.</td>
<td>35%</td>
<td>59%</td>
</tr>
<tr>
<td>2.0 PEX vol.</td>
<td>39%</td>
<td>65%</td>
</tr>
</tbody>
</table>
TPE:

• One volume exchange removes about 63%-65% of most plasma constituents

• A single two-volume exchange removes about 86% of plasma constituents

⇒ Increasing the volume beyond 1-1.5 volumes has very little impact on removal of plasma constituents
Normal Constituents Removed

Coagulation factors:

• Most coagulation factors are lost at the same rate

• Rapidly synthesized; replacement usually is 2-3 days following exchange

• Practical: measure PT/PTT/Fibrinogen every 2-3 days (rather than daily)

Platelets:

• ↓ 25-30% per procedure

• Endogenous synthesis replaces lost platelets within 2-4 days (except hypoplastic/aplastic marrow)

• Lab work (esp. chemistry): not immediate post-procedure; allow equilibrium intra/extravascular space
Apheresis Procedural Elements (+ Practical Considerations):

• Venous access
• Replacement fluid
• Normal/abnormal constituents removed
• Anticoagulation
• Patient history and medications
• Frequency and number of procedures
• Complications
Anticoagulation

Anticoagulation citrate Dextrose (ACD):
- Found in human cells, plant cells, and citrus fruits
- Chelates positively charged calcium ions (ionized calcium) and blocks calcium-dependent clotting factor reactions
- Works extracorporeally
- Metabolized in the liver almost immediately upon return
- Side effects: hypocalcemia.
  ↑ small pts, large vol. of citrated blood, liver dysfunction

Heparin:
- Prevents conversion of fibrinogen to fibrin and prothrombin to thrombin
- Systemic anticoagulation
- Metabolized slowly 1-2 hours
- Individual sensitivity and elimination rates
Anticoagulation

Citrate in Replacement Fluids

- Plasma
- Albumin
- Saline

[Bar graph showing the comparison of citrate levels in plasma, albumin, and saline]
Apheresis Procedural Elements (+ Practical Considerations):

• Venous Access
• Replacement Fluid
• Normal/abnormal Constituents Removed
• Anticoagulation
• Patient History and Medications
• Frequency and Number of Procedures
• Complications
Patient History and Medications

• Does patient have a disease which is amenable to treatment by the requested apheresis procedure

• Does the patient/donor capable of sustaining the fluid shifts associated with apheresis

• Certain medications, most notably antibiotics and anticoagulant can be removed by apheresis - should be given *immediately after* the procedure

• Angiotensin-converting enzymes (ACE) inhibitors
ACE inhibitors

A.C.E.

Angiotensin I → Angiotensin II

Vasoconstriction
ACE inhibitors

A.C.E. Inhibitor

Angiotensin I \rightarrow X \rightarrow Angiotensin II

No vasoconstrictive effect
ACE inhibitors and Apheresis

- Activation of XII
- Inhibition of Kinase II

ACE Inhibitor

Kinase I & II

Prekalikrein → Kallikrein → H.M.W.K → Bradykinin

Bradykinin Breaks down
Vasodilatation

ACE inhibitors and Apheresis
Apheresis Procedural Elements (+ Practical Considerations):

• Venous access
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• Normal/abnormal constituents removed
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• Frequency and number of procedures
• Complications
## Frequency and Number of Procedures

Depends on: Disease being treated, Patient signs and symptoms, Lab values

<table>
<thead>
<tr>
<th>Substance</th>
<th>Volume Treated (ml/kg)</th>
<th>Treatment Interval (hours)</th>
<th>Number of Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoantibodies</td>
<td>40 – 60</td>
<td>24 – 48</td>
<td>4 – 6</td>
</tr>
<tr>
<td>Immune complexes</td>
<td>40 – 60</td>
<td>24 – 48</td>
<td>treat to response</td>
</tr>
<tr>
<td>Paraproteins</td>
<td>40 – 60</td>
<td>24</td>
<td>treat to response</td>
</tr>
<tr>
<td>Cryoproteins</td>
<td>40 – 60</td>
<td>24 – 48</td>
<td>treat to response</td>
</tr>
<tr>
<td>Toxins</td>
<td>40 – 60</td>
<td>24 – 72</td>
<td>treat to response</td>
</tr>
<tr>
<td>TTP / HUS</td>
<td>40</td>
<td>24</td>
<td>to remission</td>
</tr>
</tbody>
</table>

Modified from: Weinstein, in McLeod, Apheresis, Principles and Practice, 3rd edition, AABB press, 2010
## Alteration in Blood Constituents by a 1- PV Exchange

<table>
<thead>
<tr>
<th>Constituent</th>
<th>% decrease</th>
<th>% recovery 48 hrs post exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting factors</td>
<td>25 – 50</td>
<td>80 – 100</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>63</td>
<td>65</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>63</td>
<td>45</td>
</tr>
<tr>
<td>Paraproteins</td>
<td>20 – 30</td>
<td>Variable</td>
</tr>
<tr>
<td>Liver Enzymes</td>
<td>55 – 60</td>
<td>100</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>C3</td>
<td>63</td>
<td>60 – 100</td>
</tr>
<tr>
<td>Platelets</td>
<td>25 – 30</td>
<td>75 – 100</td>
</tr>
</tbody>
</table>

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Apheresis Procedural Elements (+ Practical Considerations):

• Venous access
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• Frequency and number of procedures
• Complications
Complications

1) Hypotension

S/S: lightheadedness  \(\uparrow\) pulse rate
  dizziness
  faintness
  shallow breaths
  perspiration

Treatment: \(\downarrow\) head of bed, \(\uparrow\) foot of bed, Give NS, Monitor VS,
Look for drugs (ACE inhibitors)

2) Vasovagal syncope

S/S: \(\downarrow\) B/P  \(\downarrow\) pulse rate
  feeling of apprehension, distress, doom
  nausea, Pallor, sweating, syncope, convulsions

Treatment: same as hypotension
3) Hypocalcemia

S/S: Parasthesia, perioral tingling
   Chills/vibrations of chest wall
   Severe citrate toxicity - tetany, heart rhythm disturbances

Treatment:
- ↓AC flow rate to the patient
- Decrease blood flow rate
- Give Ca tables (Tums)
- Give dairy products
- For severe citrate toxicity – stop procedure, IV Calcium
Complications - 3

4) Allergic reaction:

Etiology: blood products/ ethylene oxide/ACE inhibitors

S/S: hives         swelling (eyes, lips, tongue)
    rash           breathing difficulties
    flushing, hypotension (m/p ACE inhibitors)
    burning eyes, periorbital edema (m/p ethylene oxide)

Treatment:

Pause procedure

Give medication per order: Antihistamines, corticosteroids, epinephrine

Discontinue procedure if no improvement
Complications – 4

5) Other side effects:

* Vascular access: hematoma, phlebitis, infection
* Air embolism
* Loss of blood components: → bleeding
* Thrombocytopenia (30% decrease)
* Hypofibrinogenemia (50% decrease)
### TABLE II. Adverse Reactions of Therapeutic Apheresis

<table>
<thead>
<tr>
<th>Reaction</th>
<th>% of procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACD Toxicity</td>
<td>3.0</td>
</tr>
<tr>
<td>Vasovagal Reactions</td>
<td>0.5</td>
</tr>
<tr>
<td>Vascular Access Complications</td>
<td>0.15</td>
</tr>
<tr>
<td>FFP Related Reactions</td>
<td>0.12</td>
</tr>
<tr>
<td>Hepatitis B (from FFP)</td>
<td>0.06</td>
</tr>
<tr>
<td>Arrhythmias</td>
<td>0.01</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>0.01</td>
</tr>
<tr>
<td>Single death (from underlying disease)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3.856</strong></td>
</tr>
</tbody>
</table>

### TABLE III. Severity of Adverse Reactions (%)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Fatal</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACD toxicity</td>
<td>85</td>
<td>12</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Vasovagal reactions</td>
<td>1</td>
<td>73</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Vascular access complications</td>
<td>39</td>
<td>49</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>FFP related reactions</td>
<td>36</td>
<td>53</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>
Therapeutic Apheresis Math

Blood/Plasma Volume

- Total Blood Volume (TBV):
  - Height
  - Weight
  - Sex

- Plasma Volume
  - TBV x (1-Hct)

Calculating the Pt’s Plasma Volume

\[ \text{TBV} \times (1-\text{Hct}) = 3600\text{mls} \]
Blood/Plasma Volume Calculations

• Calculate treatment dose
  • TPE and RBC Exchange replacement fluid volumes
  • Cytoreduction and PBSC collections

• Determine patient tolerance/safety

Calculating % of extracorporeal volume
  - The amount of blood outside the patient’s body at any given time
  - Should not exceed 15% of patient's total estimated blood volume
  - Depend on the technology/procedure, it varies between 131-284 ml
Blood Volume Calculations

**Total Blood Volume (TBV)**

- **Three Methods:**
  - Gilcher’s rule of Five for adults
  - Nadler’s formula for adults
  - Pediatrics: ml/Kg

- **Volume Adjustment:**
  - Pregnancy, Muscularity, Obesity
TBV - Nadler’s Formula

Nadler’s Formula

- Males:

\[ 0.006012 \times \text{height}^3 \text{ (inches)} + 14.6 \times \text{weight (pounds)} + 640 = \text{TBV in mLs} \]

Nadler’s Formula (cont.)

- Females:

\[ 0.005835 \times \text{height}^3 \text{ (inches)} + 15 \times \text{weight (pounds)} + 183 = \text{TBV in mLs} \]

Nadler’s Formula (cont.)

Example: Male

- height: 62 inches
- weight: 190 lb

\[ 0.006012 \times 238328 \text{ (1458)} + 14.6 \times 190 \text{ (2774)} + 604 = 4836 \text{ mL} \]
## Gilcher’s Rule of Fives

### Blood Volume (mL/kg of body weight)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Fat</th>
<th>Thin</th>
<th>Normal</th>
<th>Muscular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>60</td>
<td>65</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td>Female</td>
<td>55</td>
<td>60</td>
<td>65</td>
<td>70</td>
</tr>
<tr>
<td>Infant/child</td>
<td>-</td>
<td>-</td>
<td>70/80</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 16-2. Estimated Blood Volume

<table>
<thead>
<tr>
<th>Patient</th>
<th>Estimated Blood Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult (not pregnant)</td>
<td>70 mL/kg</td>
</tr>
<tr>
<td></td>
<td>(Estimated plasma volume: 40 mL/kg)</td>
</tr>
<tr>
<td>Pregnant female</td>
<td>80 mL/kg</td>
</tr>
<tr>
<td>Child</td>
<td>80 mL/kg</td>
</tr>
<tr>
<td>Full-term neonate</td>
<td>85 mL/kg</td>
</tr>
<tr>
<td>Preterm neonate</td>
<td>100 mL/kg</td>
</tr>
</tbody>
</table>
TBV Adjustments

- **Fat** has 11-22 ml of blood per kg
  - Obese: may use lean body weight plus 20%

- **Muscle** has 92 ml of blood per kg
  - Body builders: increase TBV

**Pregnancy**

- Plasma and RBC Volume Increases:
  - Amount of increase is dependent on the number of fetuses, number of previous pregnancies, and gestation. Plasma volume increase plateaus at 32 weeks and RBC increase plateaus at 34 weeks.
  - Plasma volume increase is greater than the RBC volume increase
    - Anemia of pregnancy!
Treatment Dosage

A “typical” order for TPE:

- **Remove 3L of plasma** (based on 1PV exchange; regular size 70kg patient; PV ~40 mg/kg)

- **Replacement fluid** per disease: for example
  - **TTP**: Replace 100% with 3L FFP (~ 12 units, 250cc each)
  - Or **GBS**: replace 100% with 3L 5% albumin (each alb. 250cc =12 bottles)

- **Frequency**: per disease: for example
  - **TTP**: daily; **GBS**: QOD x 5 treatments
Peripheral Blood Stem Cell (PBSC) Collections: Why, What, When
Hematopoietic Stem Cells Transplant

Preparative Regimen: TBI, Chemo
Role: eradicate cancer, immunosuppression to allow engraftment (allo-transplant)
## Sources of Hematopoietic Progenitors Cells

<table>
<thead>
<tr>
<th>Sources</th>
<th>Bone Marrow</th>
<th>Peripheral Blood</th>
<th>Cord Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantage</strong></td>
<td>• Large number of cells</td>
<td>• Easy to collect</td>
<td>• Collection has no risks</td>
</tr>
<tr>
<td></td>
<td>• Lower number of mature T-cells</td>
<td>• Multiple collection</td>
<td>• Readily available</td>
</tr>
<tr>
<td><strong>Disadvantage</strong></td>
<td>• Surgical procedure</td>
<td>• Treatment with G-CSF</td>
<td>• Low cell dose</td>
</tr>
<tr>
<td></td>
<td>• General anesthesia</td>
<td>• Bone pain</td>
<td>• No multiple collection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• May require central venous access</td>
<td></td>
</tr>
</tbody>
</table>

- **Bone Marrow**: Stem Cells (PBSC)
- **Peripheral Blood**: Stem Cells (PBSC)
- **Cord Blood**: Stem Cells (PBSC)
HSCs Quantification: Why CD34

CD34 remains the major surface marker for identifying early progenitors
So, we know “who and what” we need…

How do we collect the dose we need?
In Steady State

- HPCs circulating in very low concentration: CD34 is present on ~1.5% (1-3%) of the BM Cells & <0.1% of WBC in PB.
- CD34 concentration in PB is 2-5X10^6/L
- For transplant recipient 70kg - you will need 2-5X10^6/kg =140-350X10^6 CD34
- 140-350X10^6 CD34: you need to collect 2-5X10^6/L 28-175 L blood
- Apheresis machines collect 50-70% CD34 cells from the blood ⇒ 56-350 L blood would have to be processed
- Impractical, expensive and probably not possible – something has to be done…
Increase the Yield of Collection

• Increase number of CD34 cells - mobilization
• Increase volume of blood processed each collection
• Increase number of collections
Mobilization of PBSCs

- **Hematopoietic Growth Factors:**
  FDA approved: Granulocyte colony stimulating factor (G-CSF), Granulocyte/macrophage stimulating factor (GM-CSF)

- **Chemotherapy** (not for allogeneic donors)
  - HPCs ↑↑ (X20-25) during early hem. Recovery phase after chemotherapy-induced-marrow-aplasia

- **AMD3100 (Mozobil™, plerixafor)**
  - Potent and selective inhibitor of CXCR4
  - Reversible inhibition of the binding of stroma-derived factor (SDF-1α) to its receptor CXCR4
Hematopoietic Growth Factors – WBC Effects (Healthy Donors)

- WBC, gran. ↑ within 12-18H post first dose
- Usually WBC ↑ to 30-40 x10^9/L
- Gran. will stay ↑ as long as daily dose is continued
- Lymphocyte, monocyte count ↑ slightly
Hematopoietic Growth Factors – CD34 Effects (Healthy Donors)

• Do not ↑ until 3-4 daily doses are given
• Maximum ↑ after 4-5 doses
• After that- ↓ even if continue G-CSF

⇒ Window of collection is very narrow
⇒ Most centers will start collections 12-24h post 3-5 days of G-CSF injection
Hematopoietic Growth Factors – CD34 Effects – Cont.

- Therapeutic dose for 5 days: CD34 ↑ 10-30 fold (w/chemo – 50-200)
- Peak CD34 cell count on D4-5: 20-100/μL
- Wide interindividual variability

Kinetics of CD34 mobilization: G-CSF and GM-CSF

Fischmeister et al, Ann Hematol, 1999
Hematopoietic Growth Factors – CD34 Effects - Cont.

• Preharvest CD34 cell concentration in the donor’s blood is predictive of the total yield of progenitor cells

• In general, a peripheral blood CD34 cell concentration of $10 /\mu\text{L}$ can be expected to result in a yield of at least $1 \times 10^6 /\text{kg}$

• Other factors: gender ($\text{M}>\text{F}$), age ($<65$ better yield), prior chemo/radiation
So, When to start the collection?

• At least 4-5 days of G-CSF injection
• ↑ WBC: 30-40 x10⁹/L; 5-10 after chemo, Peds lower
• Preharvest peripheral blood CD34 cell concentration of at least 10 /μL
  (allo higher; auto- lower …the important thing is to set a threshold!!!)
• Range of reported triggers: 5-20 CD34+ cells/ μL
HPC Collections – Technical Aspects

- **Long procedures**
- **Extracorporeal Volume (ECV):** high with MNC sets; Should not exceed 15% of patient's total estimated blood volume
  - → **Pediatrics (<20kg)** – RBC prime
- **HPCs** are similar in size and density to lymphocytes and monocytes
  - → **HPCs** are collected with large number of lymphocytes and platelets
HPC Collection Technical Aspects Guide

COBE Spectra WBC Colorgram

- Increase Plasma Pump Rate
- Decrease Plasma Pump Rate

- 7.5% (PMN)
- 5%
- 3%
- 2%
- 1% (MNC)
- 0.5%

Approximate Hct
Post Collection Donor Issues

- **Platelets**
  - Each collection, a donor loses $\sim 4 \times 10^{11}$ plt
  - Plt count $\downarrow 30\%$ (in product + G-CSF suppression)
  - After 2 collections, plt $< 100,000$ in 20-23\% of donors
  - Delayed plt recovery (as oppose to immediate in plateletpheresis donors):
    start to rise only $\geq 2$ days
    return to normal 7-10 days post collection
    pre-donation baseline by 1 year post donation
  - Donors with low platelet counts are at potential risk from bleeding and remain at risk for up to 1 week

Miller, BBMT 2008; Tassi BMT 2005
# Collection Donor Issues
## Side Effects of Mobilizing Agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Common toxicities</th>
<th>Uncommon toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF</td>
<td>Bone pain</td>
<td>Splenic rupture</td>
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<tr>
<td></td>
<td>Low grade fever</td>
<td>Thrombosis (CVA, MI)</td>
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<tr>
<td></td>
<td>Headache</td>
<td>Flare of autoimmune disease</td>
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<tr>
<td></td>
<td>Injection site reaction</td>
<td>Precipitation of sickle cell crisis</td>
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<tr>
<td></td>
<td>Splenic enlargement</td>
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<td>Bone pain</td>
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<td>Fluid retention</td>
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<tr>
<td>AMD3100</td>
<td>Bloating, Flatulence</td>
<td>Premature ventricular contractions</td>
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<tr>
<td></td>
<td>Injection site reaction</td>
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<td></td>
<td>Paresthesias</td>
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</tbody>
</table>
Post Collection Donor Issues

G-CSF long term safety

• Available reports from single institutions with f/u for as long as 7 years have not revealed an increased risk of developing leukemia or myelodysplasia after HPC mobilization
  Cavallaro, BMT 2000; anderlini BMT 2002, Tassi BMT 2005

• F/u of 3928 unrelated donors in a single center demonstrated incidence of leukemia among donors that was similar to the expected rate in an age-adjusted control population
  Holig blood 2009

• Prospective trial of 2408 unrelated donors from NMDP – no cases of AML of myelodysplasia
  Pulsipher Blood 2009