Transfusion Support for Patients with Thrombocytopenia

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Objectives

- Explain the etiology of the different types of platelet refractoriness
- Describe how platelet refractoriness is diagnosed
- Discuss different management approaches of platelet refractoriness
Platelet Transfusion Settings

- Platelets maintain endothelial integrity & plug gaps (~7,100 /μL/d req. for the latter)
- **Therapeutic transfusion**
  - Used for WHO Gr ≥2 bleeding in the setting of actual or functional thrombocytopenia
  - Obvious end-point – cessation of bleeding
- **Pre-procedural transfusion**
  - Considered for surgical interventions and closed procedures with a risk of hemorrhage – most thresholds based upon expert opinion
- **Prophylaxis in Hypoproliferative Thrombocytopenia**
  - Due to primary or secondary (therapy related) bone marrow dysfunction
  - 50 – 75% of platelets currently transfused to prevent bleeding
  - Presumes that maintenance of counts above a “safe” threshold will result in lower hemorrhagic morbidity/ mortality

Platelet Transfusion Success

- **Bleeding**: Patient stops bleeding
- **Pre-Procedural**: Patient doesn’t bleed (or bleeds less) during surgery or invasive procedure
- **Prophylaxis**: Platelet count goes up
  - What is the right increment?
  - If the desired increment isn’t achieved, what is the cause?
Platelet Refractoriness

- **Definition:**
  - Platelet transfusion refractoriness is defined as the repeated failure to achieve satisfactory responses to platelet transfusions from random donors

- **Causes**
  - Non-Immune (sepsis, fever, medications, etc.)
  - Immune (antibodies)
  - Transfusion Service Practice
    - ABO compatibility, Irradiation, Date of unit

Platelet Refractoriness

Table I. Causes of platelet refractoriness

<table>
<thead>
<tr>
<th>Immune</th>
<th>Non-immune</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet alloantibodies</td>
<td>Infection</td>
</tr>
<tr>
<td>Human leucocyte antigen</td>
<td>High fever</td>
</tr>
<tr>
<td>Human platelet antigen</td>
<td>Antibiotics (vancomycin)</td>
</tr>
<tr>
<td>ABO</td>
<td>Anti-fungal medications (amphotericin B)</td>
</tr>
<tr>
<td>Other antibodies</td>
<td>Heparin</td>
</tr>
<tr>
<td>Platelet autoantibodies</td>
<td>Disseminated intravascular coagulation</td>
</tr>
<tr>
<td>Drug-dependent platelet antibodies</td>
<td>Bleeding</td>
</tr>
<tr>
<td>Immune complexes</td>
<td>Graft-versus-host disease</td>
</tr>
<tr>
<td></td>
<td>Veno-occlusive disorder</td>
</tr>
<tr>
<td></td>
<td>Splenomegaly</td>
</tr>
<tr>
<td></td>
<td>Increasing weight</td>
</tr>
<tr>
<td></td>
<td>Pregnancies (multiple)</td>
</tr>
</tbody>
</table>

- Study: 44% (116/266) patients with “unsatisfactory” transfusions
  - 88% non-immune
  - 25% immune
- Immune and Non-immune causes can overlap

The “Refractory” Patient

- Blood Bank get an order for “HLA matched platelets.”
  - “I’d like HLA matched platelets for my patient because……
    - ….they are better, right?”
    - ….my patient didn’t have the increase I was expecting.”
    - ….my patient is clearly refractory.”

- Further investigation of request is needed.
The “Refractory” Patient

- Determine if the increment is adequate
  - “The morning platelet count was lower today than yesterday, and the patient received platelets yesterday.”
- Measure platelet count 10-60 minutes and 20-24 hrs post transfusion on two occasions
  - Post-transfusion Platelet increment
  - Platelet recovery
  - Corrected Count Increment
Determining Cause

- Post-transfusion Platelet Increment (PPI)
  - \( PPI = \text{Post Plt count} - \text{Pre Plt count (}/L) \)
- Percentage Platelet Recovery (PPR)
  - \( PPR = \frac{\text{PPI (}/L)}{\text{Total Blood Volume x 100\%}} \times \text{platelets transfused (10^{11})} \)
- Transfusion adequate if
  - >30\% at 1 hr post-transfusion
  - >20\% at 20-24 hrs
Determining Cause

- **Post-transfusion Platelet Increment (PPI)**
  - $\text{PPI} = \text{Post Plt count} - \text{Pre Plt count} \, (/L)$

- **Corrected Count Increment (CCI)**
  - $\text{CCI} = \frac{\text{PPI}}{\text{L}} \times \text{Body Surface Area (m}^2\text{)}$ platelets transfused ($10^{11}$)

- Transfusion adequate if
  - $1 \text{ hr CCI} > 5-10 \times 10^9/L$
  - $1 \text{ hr CCI} > 5 \times 10^9/L$ if platelet count of unit is known (TRAP)

Hod, E and Schwartz, J *Br J of Haematol*, 2008 142, 348–360
Algorithm for Refractoriness

Alloimmune refractoriness?

Transfuse fresh ABO-matched platelets

Measure/calculate 10-60 min CCI (2 events)

Over threshold

Not refractory

Under threshold

Refractory! Patient needs testing to determine best products
Finding Products for the Patient

- HLA “Type and Screen”
  - Determine HLA type of patient
  - Identify HLA antibodies present

- Selecting products
  - Platelet crossmatch
  - HLA antigen matching
  - HLA antigen avoid
  - HLA epitope matching
Laboratory Testing

- Lymphocytotoxicity test (LCT)
- Platelet immunofluorescence test (PIFT)
- Lymphocyte immunofluorescence test (LIFT)
- Enzyme-linked immunosorbent assay (ELISA)
- Antigen capture ELISA (ACE)
- Monoclonal antibody-specific immobilization of platelet antigens (MAIPA)
- Solid-phase red cell agglutination test (SPRCA)
- Multiplex flow cytometric bead assays
### Table 3. HLA expressions in blood cells

<table>
<thead>
<tr>
<th></th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-Cw</th>
<th>HLA-DR</th>
<th>HLA-DQ</th>
<th>HLA-DP</th>
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<tbody>
<tr>
<td>Hematopoietic precursor</td>
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<td>+</td>
<td>ND</td>
<td>+</td>
<td>Low</td>
<td>+</td>
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<tr>
<td>cells</td>
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<td></td>
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<tr>
<td>Granulocytes</td>
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<td>+</td>
<td>ND</td>
<td>+/-0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>T cells</td>
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<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Activated T cells</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
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<tr>
<td>B cells</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>Monocytes</td>
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<td>+</td>
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<td>Platelets</td>
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<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: ND = not determined / not described.
PCR WITH SEQUENCE SPECIFIC PRIMERS

SSP-PCR

- Matched
- Mismatched

Specific Amplification
No amplification
Detection

PCR-SSOP

Sequencing: Sanger and NGS
What's on the beads: Natural antigens
Single recombinant HLAs produced by mammalian expression system
<table>
<thead>
<tr>
<th>Specificity A locus</th>
<th>Specificity B locus</th>
<th>Specificity C locus</th>
<th>Specificity DR locus</th>
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</thead>
<tbody>
<tr>
<td>A*3002</td>
<td>18.9</td>
<td>B*1512</td>
<td>Cw*1701</td>
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<tr>
<td>A*3101</td>
<td>11.3</td>
<td>B*8201</td>
<td>Cw*0202</td>
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<tr>
<td>A*8001</td>
<td>8.5</td>
<td>B*1516</td>
<td>Cw*0302</td>
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<tr>
<td>A*3401</td>
<td>6.8</td>
<td>B*3701</td>
<td>Cw*0602</td>
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<td>A*6602</td>
<td>6.6</td>
<td>B*4402</td>
<td>Cw*0303</td>
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<td>A*4301</td>
<td>5.9</td>
<td>B*4501</td>
<td>Cw*0501</td>
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<tr>
<td>A*6601</td>
<td>5.9</td>
<td>B*8101</td>
<td>Cw*0102</td>
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<td>A*0101</td>
<td>5.7</td>
<td>B*0801</td>
<td>Cw*0401</td>
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<td>A*2501</td>
<td>5.7</td>
<td>B*5401</td>
<td>Cw*1502</td>
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<td>A*3301</td>
<td>5.2</td>
<td>B*4201</td>
<td>Cw*1802</td>
</tr>
<tr>
<td>A*1102</td>
<td>4.5</td>
<td>B*5601</td>
<td></td>
</tr>
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<td>4.2</td>
<td>B*0702</td>
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<td>A*2402</td>
<td>4</td>
<td>B*5501</td>
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<td>A*2601</td>
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<td>B*5703</td>
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<td>3.5</td>
<td>B*6701</td>
<td></td>
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<tr>
<td>A*3303</td>
<td>3.5</td>
<td>B*1502</td>
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<tr>
<td>A*2403</td>
<td>3.3</td>
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<td>A*2902</td>
<td>3.1</td>
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<tr>
<td>A*7401</td>
<td>3.1</td>
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</table>

*DRB1*0404 5.4

Specificity DQ locus
DQA1*0503/DQB1*0301 10.8
DQA1*0601/DQB1*0301 10.6
DQA1*0303/DQB1*0301 9.4
DQA1*0505/DQB1*0301 8.3
DQA1*0301/DQB1*0301 6.1
DQA1*0501/DQB1*0201 3.5
DQA1*0102/DQB1*0502 3.3

Specificity DP locus
DPA1*0201/DPB1*0101 20.5

*Morales et. al., Transpl. 86:1111, 2008*
Beads in Single Antigen Beads (SAB) Assay

Intact & Heavy Chain Ag. Beads

Heavy Chain Ag. Beads

Intact Ag. Beads
Platelet Crossmatching
Immucor Capture-P™ Solid Phase Red Cell Adherence (SPRCA) assay most frequently used

Platelet Monolayer

Positive
(Incompatible)

Negative
(Compatible)

Anti-Platelet Antibody
Indicator Red Cell
Coated with Anti-Human IgG
<table>
<thead>
<tr>
<th>Patient→</th>
<th>HLA Antigen: A1, A2, B7, B8</th>
<th>HLA Antibody: B44, B60</th>
<th>Product Status: Quarantine/Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor 1</td>
<td></td>
<td>A2, B7</td>
<td>R</td>
</tr>
<tr>
<td>Donor 2</td>
<td>A3, A68, B27, B57</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Donor 3</td>
<td>A1, A2, B7, B44</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Donor 4</td>
<td>A2, A3, B60, B62</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Donor 5</td>
<td>A23, A33, B38, B59</td>
<td></td>
<td>Q</td>
</tr>
<tr>
<td>Donor 6</td>
<td>A1, A2, B7</td>
<td></td>
<td>Q</td>
</tr>
<tr>
<td>Donor 7</td>
<td>A1, B7, B8</td>
<td></td>
<td>Q</td>
</tr>
<tr>
<td>Donor 8</td>
<td>A29, A32, B35 (HPA-1b)</td>
<td></td>
<td>Q</td>
</tr>
</tbody>
</table>
Platelet Crossmatching

- Detects HPA as well as HLA Ab, but also strong ABO Abs
  - 2/3\textsuperscript{rd}s of O’s have anti-A,B titers high enough to invalidate test
- General correlation with the PRA, but misses some significant IgG & all IgM antibodies
  - Up to 17\% of significant AHG-CDC-detected antibodies may be missed in solid phase testing (i.e., product inappropriately appears compatible)
- Only 475 of 2800 (17\%) XMs compatible in 1 large study
- Crossmatch availability issues
  - Limited or no night & weekend availability; 4-6 hour test TAT (one ties up distributable units during testing or loses them)
- Less expensive only for very brief support or in low-PRA patients

Antigen-Negative Approach

- Antibody Specificity Prediction (1621 tfxns. in 114 pts.)
  - PPRs adjusted for ABO, post-count timing, PRA, fever & ampho B use
  - Post-tfxn. PPR: ASP - 24.13% XM - 23.38% vs. HLA - 20.77% Rnd - 14.87%
- Markedly expands # of potential donors
  (n=29 recipients vs. 7247 donors)

<table>
<thead>
<tr>
<th></th>
<th>A matches</th>
<th>BU matches</th>
<th>ASP matches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>0 - 60</td>
<td>0 - 117</td>
<td>11 - 4638</td>
</tr>
<tr>
<td>Median/Mean</td>
<td>1 / 6</td>
<td>20 / 33</td>
<td>1365 / 1426</td>
</tr>
</tbody>
</table>

- Perhaps as good as identical matches…

HLA Antibody Identification

Luminex-Based Antibody Identification

Lambda Array Beads Multi-Analyte System (LABMAS)
LABScan™ 100 Flow Analyzer, Luminex XY 96-sample reader and:
LABScreen® Single Antigen Beads
(~30 HLA-A / ~50 HLA-B Beads)

Uses PE-conj. goat **anti-human IgG** to detect antibody binding to beads (or PE-conj. C1q)
Antigen-Negative Approach (cont’d)

- Some HLA antigens with low platelet expression may yield good increments despite the presence of specific Ab
  - B8
  - B12 (44,45)
  - B13 [variable Bw4 expression]
  - B14 (64,65) [variable Bw6 expression]
- Need 1-hr post-transfusion counts to assess quality of each Ag-positive donor

### HLA-Based Selection

- **Duquesnoy HLA-A & -B locus match determines outcome**
  - **A** HLA identical all 4 loci  [A1 A2 B7 B8]
  - **BU** All loci identical, only 3 Ag’s detected  [A1 B7 B8]
  - **B2U** All loci identical, only 2 Ag’s detected  [A1 B8]
  - **BX** 3 loci identical, 4th cross-reactive  [A1 A11 B7 B8]
  - **BUX** 3 Ag’s, 2 identical, 3rd X-reactive  [A1 A11 B8]
  - **B2X** 2 loci identical, 2 X-reactive  [A1 A11 B7 B60]
  - **C** one antigen mismatched  [A1 A32 B7 B8]
  - **D** two or more antigens mismatched, at least one NOT X-reactive  [A1 A32 B7 B60]

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Computer-Assisted HLA Selection

- Duquesnoy abandoned his older CREG-matching schema for one more data-driven: **HLAMatchmaker**
- Each HLA antigen has shared immunogenic amino acid epitopes ("triplets" & "eplets"); patients cannot produce alloAb to their own HLA-A, -B & -C epitopes
  - Donors without mismatched eplets are presumably fully compatible (A/BU-like)
  - Inputting HLA type identifies alloantigens with the fewest mismatched eplets (i.e., BX/C matches more likely to succeed)
    - With older triplet-based version, ≥ 9 mismatches required before CCI was compromised; eplet version ≥ 11 mismatches
- Inputting results of the Ab ID, becomes a marginally-enhanced automated antigen-negative selector
  - A/BU success rate ~85%, old CREG matching ~63% and enhanced HLAM ~84% 1-hr CCIs ≥ 7,500

Structurally Based HLA Matching

- Linear sequences of 3 Amino Acids (AA triplets) in antibody-accessible positions on HLA molecules
- Epitope—part of an antigen that is recognized by the immune system → antibody defined.
- Eplets—AA residues about 3 Å apart from each other and at least one of them is non-self → HLA ‘Epitope’ repertoire.
- Eplets can be identical to those referred to as triplets but others have residues in discontinuous sequence positions that cluster together on the molecular surface.
Epitope-Based Matching

- HLA-A, -B, and -C allele resolution typing of patients and donors
- HLA antibody analysis on the patient by complement-dependent methods and single antigen bead
- HLA Matchmaker-based analysis to identify acceptable mismatches
- Conduct donor search in allele typed PLT donor registry with an HLA Matchmaker-based search engine;
- Enter the HLA type of the patient and the nonreactive mismatched alleles in this database for a list of donors with matches and acceptable mismatches at the eplet level
- No need for PLT cross-match testing for HLA incompatibility.
- Minimal numbers of mismatched eplets:
- Avoid immunogenic eplets.
HLA Tests

- Platelet crossmatch, 4 hour TAT, morning sample receipt, a single red top
- HLA-A/B typing, 6 hour TAT for STAT, 24-48 hour TAT for regular request, a single EDTA or 2 swabs
- HLA antibody analysis, 6-8 hour TAT for STAT, 24-48 hour TAT for regular request, a single red top
Practical Selection of Products

- Information known about patient
- Options available from blood center
  - Testing performed on platelet donors
  - Pool of tested donors
- Urgency of need (Bleeding or not)
  - Non Urgent (not bleeding)
  - Urgent (low count or risk of bleeding)
  - STAT! (Bleeding)
Selection: Patient Information

- Tested for HLA Abs
  - yes: HLA Abs present
    - yes: Crossmatched, Ag neg or HLA matched Plts
    - no: Crossmatched or Ag negative Plts
  - no: Crossmatched Plts

- Tested for HLA Type
  - yes: Crossmatched or Ag negative Plts
  - no: Positive; Ag neg Plts

- HLA Abs present
  - yes: Crossmatched, Ag neg or HLA matched Plts
  - no: HPA-1a neg Plts

- HPA Abs present
  - yes: HPA-1a Abs present
  - no: Crossmatched Plts

STOP Non-immune

Non Urgent Need

- Schedule an HLA matched donor
  - Blood center identifies a donor match
  - Donor scheduled for a donation
  - Ideal if patient can be put on transfusion schedule and enough donors available
  - Best TAT is 48-72 hrs (could be longer)
- Review currently scheduled donors
  - Matched donor may already be scheduled
  - TAT closer to best above
Urgent need

- Patient platelet count low or at risk of bleeding
- Review Blood Center Typed Inventory
  - Products available
    - Work in Progress (WIP)
    - Available for shipping
    - Just shipped – can possibly transfer
- Crossmatch Units
- TAT: Few minutes to 24 hours
STAT!

- **Patient Bleeding**
  - Random unit from shelf
  - Best match
    - Least incompatible by crossmatching
    - Typed unit that is “close enough”
      - Consider patient test results and reactivity

- **Pooled RDPs**
  - Rationale: one donor in pool may be compatible
  - High PRA patients
  - Likely not more effective than random SDP
Platelet Selection Algorithm

- If all patient testing has been performed, use Urgency Algorithm to select best product
- If testing is incomplete, product selection will be limited to options allowed with limited information
- Timely patient testing will allow more options for transfusion
Should Patients Receive HLA/HPA-Selected or Crossmatch-Selected Platelets?

**Recommendation 8**
- Patients with hypoproliferative thrombocytopenia who are refractory to platelet transfusions and have class I HLA antibodies should probably receive class I HLA-selected or crossmatch-selected platelet transfusion to increase the platelet count (weak level of evidence, weak recommendation).

**Recommendation 9**
- Patients with hypoproliferative thrombocytopenia who are refractory to platelet transfusions and have HPA antibodies should probably receive HPA-selected or crossmatch-selected platelet transfusion to increase the platelet count (very weak level of evidence, weak recommendation).
Should Patients Receive HLA/HPA-Selected or Crossmatch-Selected Platelets?

- **Recommendation 10**
  - Patients with hypoproliferative thrombocytopenia who are refractory to platelet transfusions solely due to nonimmune factors should probably not receive HLA-selected or crossmatch-selected platelets (weak level of evidence, weak recommendation).

- **Recommendation 11**
  - Patients with hypoproliferative thrombocytopenia who are not refractory to platelet transfusion should probably not receive HLA-selected, HPA-selected, or crossmatch-selected platelets (weak level of evidence, weak recommendation for HLA selection and crossmatch selection, very weak level of evidence and weak recommendation for HPA selection).
What is the best method?

- Evidence for use of HLA-matched or crossmatched platelets
  - Studies are limited
  - Not powered to detect differences in mortality and bleeding outcomes
- However, HLA-matched strategies continue to be the standard of care in many centers

Management Pearls for Refractory Pts.

- 1-hr CCIs very important in some patients
  - Clue to broadening of alloimmunity in patients requiring daily tfxns.
  - Identifies good donor-recipient (mis)matches for high PRA patients
- Establishment of a transfusion schedule is critical for recruitment of A/BU matches
  - Minimum of 3 days to get a recruited product to the hospital
- Does the pt. *really* need CMV neg. units or will LR do? (more leeway for bleeding patients than mere prophylaxis)
- All HLA-selected / crossmatched products should be irradiated to avoid TA-GvHD
- Matching is usually not helpful for patients without demonstrable HLA (and HPA) antibodies
  - Consider brief support if IgM HLA antibodies suspected
  - May succeed just because units are fresher & ABO-matched
Summary

- Platelet refractoriness – determined by carefully measuring response to platelets
- Patient testing
  - Helps determine the cause of refractoriness
  - Helps provide more options for transfusion
- Blood Center testing of donors is important in order to provide the best units for patients
Transfusion Practice Guidelines

The *Compendium* includes:
- Guidelines for blood product use
- Information about testing
- Adverse reactions

[www.redcrossblood.org](http://www.redcrossblood.org)

Go to the “For Hospitals” section

*Educational Resources* link

**Now Available as an App!!**

**SUCCESS website**
- In-depth courses on Blood Banking
- Category 1 CME credit