Strategies for Platelet Support of the Refractory Patient

Patricia M. Kopko, MD
Clinical Professor of Pathology
Overview

- Challenges
- Changes in HLA Testing
- Antibody Specificity Prediction Method
- Maximizing Quality of HLA Match
- Cross-Matched Platelets
- Complement (C1q) Fixing Solid Phase Screening
- Epitope Based Matching
2013 ASHI/AABB Joint Program Laboratory Participant Survey

• What do you think is the biggest obstacle to providing appropriate platelets for platelet refractory patients

  – Education of physicians regarding when product are needed (7)
  – Limited number of HLA typed donors (5)
  – Time/cost/logistics (4)
  – Availability of crossmatch kits (3)
2013 ASHI/AABB Joint Program Laboratory Participant Survey

• What is the one thing you wish your hospital customers understood about providing platelets for refractory patients?

  – It takes time to obtain special platelet products (10)
  – It is important to obtain post-transfusion platelet counts (5)
  – There are other causes of platelet refractoriness (3)
Biggest Obstacles to Providing Appropriate Platelets to Refractory Patients

- HLA matched platelets are often perceived as a magic bullet
- “Extra testing” can often help identify better (compatible) platelet products
- Hospitals don’t want to pay for “extra testing”
HLA System and HLA Testing
Systems of Compatibility in Humans

• ABO
• Rh
• HLA = Human Leukocyte Antigen
  – Located in the Major Histocompatibility Complex
Major Histocompatibility Complex

- Located on short arm of chromosome 6
- Usually inherit one intact MHC from each parent
- Works with T cell receptor in recognition of self vs non-self
HLA molecules

- comprised of four regions
  - Cytoplasmic region
  - Transmembrane region
  - Highly conserved region
  - Highly polymorphic peptide binding region (groove)
• Antigen Binding Groove
  • Two alpha helices form wall
  • Eight beta sheets form floor
• Comprises the most variable regions of the MHC molecule
Expression of HLA Antigens

- **Class I antigens (A, B, Cw)**
  - Expressed on all nucleated cells
  - Not expressed on red blood cells
  - Platelets come from megakaryocytes, which have a nucleus

- **Class II antigens (DR, DQ, DP)**
  - Expressed on B lymphocytes, macrophages, monocytes, dendritic cells, kupfer cells, other (activated) cells of the immune system
“HLA Matched”

- 4 of 4 Match
  - A, B (used for platelet refractoriness)

- 6 of 6 Match
  - A, B and DR loci are matched

- 8 of 8 Match
  - A, B, DR and DQ loci are matched

- 10 of 10 Match (Bone Marrow Transplant)
  - A, B, C, DR and DQ loci are matched
HLA Nomenclature

- **Hyphen** used to separate gene name from HLA prefix
- **Suffix** used to denote changes in expression
- **Separator**
- **Field Separators**
- **Field 1**: allele group
- **Field 2**: specific HLA protein
- **Field 3**: used to show a synonymous DNA substitution within the coding region
- **Field 4**: used to show differences in a non-coding region
## HLA Matched Platelets (Standard Definition)

<table>
<thead>
<tr>
<th>Match Grade</th>
<th>Description</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4 Ag match</td>
<td>A1, 3; B8, 27</td>
</tr>
<tr>
<td>B1U</td>
<td>1 Ag unknown or blank</td>
<td>A1, -; B8, 27</td>
</tr>
<tr>
<td>B1X</td>
<td>1 cross-reactive Ag</td>
<td>A1, 3; B8, 7</td>
</tr>
<tr>
<td>B2UX</td>
<td>1 Ag blank and 1 cross-reactive</td>
<td>A1, -; B8, 7</td>
</tr>
<tr>
<td>C</td>
<td>1 mismatched Ag</td>
<td>A1, 3; B8, 35</td>
</tr>
<tr>
<td>D</td>
<td>2 or more mismatched Ags</td>
<td>A1, 32; B8, 35</td>
</tr>
<tr>
<td>R</td>
<td>Random</td>
<td>A2, 28; B7, 35</td>
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</table>
Modern HLA Technologies Effect on the Definition of “HLA Matched”

- A B1U HLA matched platelet should be equivalent to an A HLA matched platelet
- A B1X matched platelet can be equivalent to a C HLA matched platelet
  - Patients often make antibodies to cross-reactive antigens.
  - If a patient is HLA A1, 3; B8, 27 and has HLA B7 antibody, then a B1X match is really a C match.
Changes in HLA Testing – HLA Antigens

• When the standard definition of HLA Matched Platelets was developed, most HLA typing was performed by serology
  – HLA typing was cell based
  – HLA typing sera was not available for all HLA antigens
  – Cross reactivity of HLA antigens limited accuracy of HLA typing

• HLA typing is now performed by molecular methods
  – PCR-SSP, SSPO, sequencing
  – Low resolution typing is typically used for platelet refractoriness testing and solid organ transplantation (example HLA-A02)
  – High resolution typing is typically used for bone marrow transplantation (example HLA-A*02:01)
  – Molecular typing is faster and less prone to ambiguity
Changes in HLA Testing – HLA Antibodies

• What used to take days to weeks, now takes hours
• HLA antibody testing used to be performed by serology
  – The patient’s serum was tested against a panel of lymphocytes on frozen cell trays
  – There was no standard panel. Every lab developed their own panel
  – Since each cell on the panel expressed up to four HLA antigens, it could be very difficult to determine antibody specificity
  – The PRA (panel reactive antibody) was the percent of positive wells in a panel. If 5 wells were positive in a 50 cell panel the PRA was 10%
  – The PRA of a single patient varied between labs because there was no standard panel
Changes in HLA Testing – HLA Antibodies

- HLA antibody testing is now performed by solid phase methods.
  - ELISA
  - Luminex
- Although the panel of antigens present in a test has not been standardized, the calculation of the PRA has been standardized.
- The cPRA (calculated Panel Reactive Antigen) is now based on antigen frequencies in the deceased donor population.
- The UNOS calculator is available at: https://optn.transplant.hrsa.gov/resources/allocation-calculators/cpra-calculator/
HLA Antibody Testing - ELISA

ELISA

Enzyme labeled AHG

Antigen

Microtiter well
HLA Antibody Testing - Luminex

Bead Assay

Fluorochrome labeled AHG

Purified antigen

Microsphere

Luminex microspheres
HLA Antibody Testing - Luminex

Luminex Analysis
Changes in HLA Testing – Effect on Platelet Refractory Work-Ups

- The entire concept that you had to prove the patient was refractory to platelet transfusion by testing their platelet count within 10 – 60 minutes after transfusion was based upon the fact that it was so time consuming to get HLA antigen and antibody testing and the testing was not very accurate.
- HLA testing has changed. We need to adjust how we think about how we approach testing in this patient group.
- Testing for HLA antibodies in this patient group is now diagnostic.
Antibody Specificity Prediction Method
Antibody Specificity Prediction (ASP) Method

• The patient is HLA A and B typed
• The specificities of the patient’s HLA antibodies are determined
• Platelets lacking these antigens are chosen
Antibody Specificity Prediction (ASP) Method

- Petz et al. studied 1621 platelet transfusions
- The percent platelet recovery (PPR) was similar in HLA-matched, cross-matched and ASP-selected transfusions
- The PPR was significantly decreased for randomly selected transfusions

Antibody Specificity Prediction (ASP) Method

- In a file of 7,247 platelet donors, there were a mean of 6 HLA-A matched donors, 33 HLA-BU matched donors and 1,426 donors identified by ASP method.
- HLA antibodies were identified by cytotoxic methods.
Modified ASP Method

- The patient is typed for HLA A and B antigens
- The patient is tested for HLA class I antibodies
- The patient’s HLA type and HLA class I antibodies are entered into a computer that searches for compatible donors.
- All platelet donors with HLA antigens corresponding to the patient’s antibodies are excluded from the list of compatible donors (incompatible platelet donors are automatically excluded)
Modified ASP Method

- If the patient’s HLA type is HLA-A1, 3; B8, 27 and the patient has an HLA-B7 antibody, all donors with HLA-B7 will be excluded, even though this is a cross-reactive antigen
- This is the same method used by the United Network for Organ Sharing for allocation of renal allografts from deceased donors in the United States
- Preferred method of finding compatible platelets for patients refractory to platelet transfusion at UCSD
HLA Matched Platelets
HLA Matched Platelets

- Typically will only need to use standard definition HLA matched platelets if the patient’s cPRA is >90%
- The blood center will often let the transfusion service know when they need to use standard definition HLA matched platelets
- This product can take much longer to obtain because usually a specific donor needs to be recruited
- For elective procedures it can be a good idea to begin the process of obtaining platelets at least a week before the procedure
Tips for Maximizing Quality of HLA Matched Platelets

- Always determine the match grade of the platelets your patient is receiving
- Always test the patient for HLA antibodies
- Always try to have a post-transfusion platelet count performed
- If a donor has a good response to a specific platelet donation, the blood center can often have the donor return and collect two or three apheresis platelet components at one time
Tips for Maximizing Quality of HLA Matched Platelets

• Some patients (not all) respond better to ABO identical platelets
• HLA-B44 and B45 are weakly expressed on platelets – incompatible transfusions expressing these antigens may provide an acceptable response
• If nothing seems to work consider ordering HPA antibody testing
Crossmatched Platelets
Platelet Crossmatch – Solid Phase

SPRCA (solid phase red cell adherence)
Intact platelets bound to microtiter plate. Antibody detection by AHG binding to sensitized red blood cells.
Platelet Crossmatch – Flow Cytometry

Platelet Crossmatch Techniques

- **Flow**
  - Washed intact platelets. Ab detection by FITC-labeled anti-IgG.
Crossmatched Platelets

- Typically available quicker than HLA matched platelets, especially if the patient has not been HLA typed
- If all platelets are compatible, the patient most likely does not have antibodies (HLA or HPA)
- Can help detect HPA antibodies if HLA antibodies are not present (HLA antibody testing is negative, some platelets are incompatible)
- Post transfusion platelet counts should be performed
- Preferred method at UCSD if:
  - Patient has anti-platelet antibodies
  - HLA antigen and antibody testing has not been completed yet
Crossmatched Platelets

- Retrospective study of 50 patients who had two or more platelet crossmatches, platelet antigen typing and platelet antibody testing
- Percent cPRA correlated well with percent positive platelet crossmatches

Crossmatched Platelets

- Study of 71 platelet refractory patients
- Received 738 crossmatch compatible platelets
- Adequate post-transfusion platelet counts were available for 443 transfusions
- CCI = 7000 ± 7900 for crossmatched platelets compared to CCI = 710 ± 2700 for random donor platelets (p < 0.001)
- Mean percent reactivity in initial (58.6%) vs final (55.3%) showed no evidence of progressive alloimunization

Wiita and Nambiar. Transfusion 2012;52:2146-54
Options for Highly Immunized Platelet Refractory Patients

- Are all antibodies identified by solid phase assays clinically significant?
- Do all antibodies identified by solid phase assays bind complement?
Complement (C1q) Fixing Solid-Phase Screening for HLA Antibodies

Detection by PE Conjugated Anti-C1q

1. Add C1q to HI serum sample
2. Add HLA antigen coated beads
3. Add PE conjugated anti-C1q
4. Wash
5. Read with LABScan™100

Legend:
- Sample antibody
- C1q
- HLA antigen coated beads
- PE conjugated anti-C1q

UC San Diego Health System
Complement (C1q) Fixing Solid-Phase Screening Increases the Availability of Compatible Platelets

- 13 highly refractory patients received 177 platelet transfusions
- Retested using C1qSAB method

<table>
<thead>
<tr>
<th></th>
<th>IgG-SAB</th>
<th>C1q-SAB</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cPRA</td>
<td>94%</td>
<td>60%</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Fontaine MJ et al, Transfusion. 2011
Complement (C1q) Fixing Solid-Phase Screening Increases the Availability of Compatible Platelets

- CCIs in compatible vs incompatible (by C1q assay) were compared
- 75% of platelets previously considered incompatible were actually compatible
- Mean CCI of compatible transfusions was 10.6 X 10^9/L
- Mean CCI of incompatible transfusions was 2.5 X 10^9/L
  - p < 0.0001
Epitope Based Matching (EBM)

- HLA antigens have multiple epitopes that can be recognized by antibodies
- Recipient’s own HLA antigens represent a repertoire of self antigens
- Antibody is not made against self epitopes
- Computer algorithm compares epitopes of patient’s HLA antigens and antibodies to donors’ epitopes to determine compatibility (program available at www.hlamatchmaker.net)
Epitope Base Matching

- Nine alloimunized patients prospectively enrolled into study
- 142 single-donor platelet transfusions evaluated
- Patients received platelets matched by A/BU method or CREG or EBM methods

## Epitope Based Matching

<table>
<thead>
<tr>
<th>Platelet Matching Method</th>
<th>A/BU</th>
<th>CREG</th>
<th>EBM</th>
</tr>
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<tbody>
<tr>
<td>Number of Transfusions</td>
<td>61</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>CCI (median)</td>
<td>14.55</td>
<td>10.12</td>
<td>22.03</td>
</tr>
<tr>
<td>Success Rate (CCI &gt; 7.5/L)</td>
<td>85.2%</td>
<td>63.2%</td>
<td>83.7%</td>
</tr>
</tbody>
</table>
Epitope Based Matching

- Patients only received CREG or EBM method platelets if A/BU matched platelets were not available
- Use of platelet matchmaker can increase available matched donors without decreasing success rate
Algorithm for New Request for Platelet Refractory Patients

Has the pt been tested for HLA Abs?  
   no  
      Start with XMed plts  
   yes  

Has the pt been HLA typed?  
   no  
      Start with XMed plts  
   yes  

Does the pt have HLA Abs?  
   no  
      Start with XMed, Ag Neg or HLA matched plts  
   yes  

Discuss availability of HPA-1a negative platelets with blood supplier  
   yes  
      Start with XMed plts  
   no  

Does the pt have HPA Abs?  
   no  
      Start with XMed plts  
   yes  

Are HPA Abs, anti-HPA 1a?  
   no  
      Start with XMed plts  
   yes  

Stop
Conclusions

• Advances in histocompatibility testing and analysis can be used to improve
  – Quality of platelet match
  – Number of well matched donors
• B1X matched platelets may really be C or one antigen mismatched platelets
• Clinical correlation and communication with blood services provider is crucial in supporting platelet refractory patients
Questions