HLA Alloimmunization: Clinical and Laboratory Aspects of Management

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Transfusion Medicine/Blood Banking Fellow
Overview

• Show clinical workup with a case example

• Examine laboratory aspects of immune mediated platelet refractory workup

• Focus on HLA alloimmunization
HPI

• 55-year-old woman with AML refractory to induction with 7+3
• Transferred from Great Falls, Montana
  – Diagnosed with AML 6/19/2012
  – FLT3-ITD+ (also FLT-D835+); Normal Cytogenetics
  – Received 7+3 in MT (6/19-6/29)
  – 14 Day Marrow (7/6): 69% blasts
• Presented to SCCA outpatient on 7/24
  – WBC 55K (7/19)
  – Plt 21 (7/24)
• Admitted to UWMC on 7/25
  – New onset fever and intermittent flashes of light
  – WBC 80K (ANC 1210), Plt 12
  – No bleeding
Social History/Medications

• Social History
  – Lives with husband in Great Falls, MT
  – Has one son in Seattle, one daughter in Anchorage

• Home medications
  – acyclovir 400mg PO BID
  – compazine 10mg PO q6hr
  – levofloxacin 750mg PO daily
  – posiconazole 200mg PO TID
  – KCl 20 meq PO daily
  – senna
  – docusate

• Inpatient medications
  – Acyclovir 800mg PO Q12 Hours
  – Allopurinol 300mg PO Daily
  – cefTAZidime 2g IVPB Q8 Hours
  – Cytarabine 193 mg IVPB Q24 Hours
  – Decitabine 39 mg IVPB Q24 Hours
  – Docusate 100mg cap 2 cap PO BID
  – Fluconazole 200mg PO Daily
  – Hydroxyurea 1,500 mg PO Q8 Hours
  – Idarubicin HCL 23.2 mg I.V. Push (non-std) Q24 Hours
  – Ondansetron 8 mg IV Q24 Hours
  – Senna 8.6mg tab 17.2 mg PO Daily
Physical Exam

- **VS:** T 38°C, P 107, R 18, BP 125/77
- **Gen:** well appearing, lying in bed in NAD,
- **Eyes:** small pupils but ERL
- **Ears/Nose/Mouth/Throat:** alopecia, no oral lesions, mmm
- **CV:** RRR, no m/g/r
- **Resp:** CTAB
- **GI:** active BS, non-distended, no rebound/guarding, slight tenderness to deep palpation RLQ and LLQ
- **Musk:** no extremity edema
- **Skin:** no rashes
- **Lymph:** no LAD
- **Neuro:** CN II-XII grossly intact, full strength, hyperreflexic in left knee
- **Psych:** AAOx3. Conversation appropriate.
Plan

• Admit patient for neutropenic fever
• Re-induction chemotherapy with 7+3
• See if eligible for FLT3 inhibitor trial
Platelet Refractory

• Standard Threshold (10K)

<table>
<thead>
<tr>
<th>Date</th>
<th>Pre-transfusion</th>
<th>Post-transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/26</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>7/27</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>7/28</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

• Immune Refractory?
Causes of Platelet Refractoriness

• Non-immune
  – Splenomegaly
  – Bleeding
  – Fever
  – Infection/Sepsis
  – DIC
  – Medications

• Immune
  – HLA
  – Platelet Specific Antigens
  – Blood Group Antigens (ABO)
Step 1: Look for Possible Non-immune Causes

- **Splenomegaly**: Possibly
  - No mention on physical exam
  - Later abdominal CT on 9/4 shows splenomegaly (15 cm)
- **Fever**: Yes; spiking fevers to 39.3°C
- **Infection/Sepsis**: No localizing signs
- **DIC**: No
- **Bleeding**: No
- **Medications**: Yes; cephalosporin & antifungal
Distinguishing Immune and Non-immune Causes

- Need Additional Data
  - 1-hour post transfusion platelet count
  - Date/Time of pre transfusion platelet count
  - Type of platelet unit transfused
    - Apheresis vs. Pooled
    - ABO Type of Unit*

- Calculate Corrected Count Increment (CCI)

Two patterns can be seen in refractory patients. A normal increment at one hour following transfusion with return to the baseline count within 24 hours (green curve) is typical of the shortening of platelet survival seen with sepsis, hematopoietic cell transplantation, disseminated intravascular coagulation, and possibly in bleeding patients and those taking medications that interfere with platelet survival. The second pattern consists of little or no increment in platelet count, even within one hour of transfusion (red curve); this pattern is seen with alloimmunization.
Step 2: Corrected Count Increment (CCI)

- Adjusts observed raw increment in plt count for plt content of transfusion and BSA of pt
- Measured within 10 min – 1 hr post transfusion
- > 5,000 pts/microL
- Need two failed CCIs

\[
CCI = \frac{\text{Platelet count increment/μL} \times \text{BSA (m}^2\text{)}}{\text{No. of platelets transfused} \times 10^{11}}
\]

Example: If a man with BSA 2.0 m\(^2\) increases his platelet count from 6,000/μL to 46,000/μL after receiving 4.0 (\(\times 10^{11}\)) platelets, then

\[
CCI = \frac{(46,000 - 6,000) \times 2.0}{4.0} = 20,000
\]

Figure 13-2. Calculation of the corrected count increment (CCI). BSA = body surface area.
## Patient’s CCIs

<table>
<thead>
<tr>
<th>Date</th>
<th>Pre transfusion</th>
<th>Type of unit</th>
<th>1-hr Post transfusion</th>
<th>CCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/26</td>
<td>8</td>
<td>4-pool</td>
<td>N/A</td>
<td>-</td>
</tr>
<tr>
<td>7/27</td>
<td>9</td>
<td>4-pool</td>
<td>14</td>
<td>4.4</td>
</tr>
<tr>
<td>7/28</td>
<td>8</td>
<td>4-pool</td>
<td>5</td>
<td>-2.6</td>
</tr>
</tbody>
</table>
The most likely causes of Immune Refractoriness

• HLA alloimmunization
  – Pregnancy
  – Prior transfusions

• Platelet Specific Antigens
  – Less likely
  – 8% of TRAP patients became positive

• Blood Group Antigens
  – PSBC gives in-group platelets
  – Expressed at a very low level on platelets
Step 3: Panel Reactive Antibodies (PRA)

- Screen for HLA alloimmunization
- Analogous to the “Screen” in a Type & Screen for Red Cell alloimmunization
- Methodologies
  - Complement-Dependent Cytotoxicity (CDC) Assay
  - FlowPRA
  - Luminex-based Bead assay
CDC Assay

Panel Reactive Antibody Assay for Cytotoxic Anti-HLA Antibodies

HLA-Typed Lymphocyte Panel (50 Individuals) + C-FDA (Fluorescence dye) (15 min)

+ Serum (30 min) + Complement (2-3 hrs)

Anti-HLA Abs

HLA Ag

Cell Lysis: (−)fluorescence

Count stained cell number Under the microscope

= 34/ total 50 (PRA 68%)

Source: Am J Transplant © 2006 Blackwell Publishing
CDC Assay

• Advantages
  – Relatively inexpensive assay
  – Easy to perform

• Disadvantages
  – Dependent on cell viability
  – Can be difficult to assign antibody specificity for highly sensitized patients
PRA Positivity

- >20% commonly used as the cut-off
- Historically refers to CDC assay
- PSBC uses a calculated PRA (cPRA) with the Luminex Analyzer
Luminex®
Uniform-Sized Microparticles

Each bead has multiple copies of a single HLA antigen
Dual-colored Beads

- Each bead has a particular ratio of green:red
- The luminex can identify each bead

2 Color Suspension Array

Fluorescence 1

Fluorescence 2
38 Beads Separated in 2-D
Luminex Single Antigen Assay

- Beads are incubated with patient serum and a PE-tagged anti-IgG antibody

- The sample is washed and analyzed using a Luminex Analyzer

- The Luminex Analyzer can identify which individual beads are positive
Sensitized to One Antigen
Highly Sensitized
Step 5: Obtain Patient’s HLA Type

- PSBC HLA lab will reflex HLA typing for positive PRAs

- SCCA patient’s usually already have an HLA type on file
## Patient’s PRA & Antibody Results

### HLA Typing

<table>
<thead>
<tr>
<th></th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-Bw</th>
<th>HLA-C</th>
<th>DRB1</th>
<th>DRB3/4/5</th>
<th>DQB1</th>
<th>DPB1</th>
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<tbody>
<tr>
<td></td>
<td>02</td>
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<td>51</td>
<td>58</td>
<td>4</td>
<td>07</td>
<td></td>
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</table>

### Recipient’s Alloantibody Testing

<table>
<thead>
<tr>
<th>Class</th>
<th>PRA</th>
<th>Unacceptable Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>99% (07/28/12)</td>
<td>A01,03,23,24,25,26,32,33,34,43,66,68,69,80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B07,08,18,27,35,37,38,39,41,42,44,46,47,48,49,50,54,55,56,59,60,62,63,67,71,72,73,75,76,77,81,82</td>
</tr>
</tbody>
</table>

**COMMENTS:**

Report amended to include HLA-B41 as an unacceptable antigen.
Donor Volunteer Recruiting

• Specific Group, who enlists donors
• Uses both Luminex results *and* HLA typing to find units for patient
• Antibody Specificity Prediction
  – Luminex results can help find units (and donors), who have antigens to which patient has not developed antibodies
  – Widens potential units available to patient
  – Does not require HLA typing
## Transfusions

<table>
<thead>
<tr>
<th>Date</th>
<th>Pre-Platelet Count</th>
<th>1 h Post-Platelet Count</th>
<th>CCI</th>
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<tbody>
<tr>
<td>7/30/2012</td>
<td>2</td>
<td>36</td>
<td>22.0</td>
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<tr>
<td>8/2/2012</td>
<td>9</td>
<td>23</td>
<td>9.1</td>
</tr>
<tr>
<td>8/6/2012</td>
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<tr>
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<tr>
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<tr>
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**Oct 9, 2012**

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<td>Class I</td>
<td>69% (10/09/12)</td>
<td>A01,25,66, B13,27,44,45,60,61,76,81,82</td>
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**COMMENTS:**

HLA antigens to avoid have been updated based on testing with this patient's new sample. Her antibodies have changed markedly since two months ago. The current unacceptable antigens should make finding suitable platelet donors easier than before, but if poor increments follow, don't hesitate to contact us for additional advice.
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**COMMENTS:**
Positive for IgG antibodies to class I HLA. Avoid using platelet donors having HLA identified as unacceptable.
Conclusion

- Patient did well with HLA matched platelets
- However, failed three more rounds of salvage chemotherapy
- Died in January of 2013