Platelets for the Refractory

Climbing the Pyramid

April 2016

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Right Method and the Right Target
Definition of Refractoriness

- Platelet transfusion – 5000-7000 platelets/ul/unit wb platelets in 70kg. man
- Apheresis – 30,000-40,000/ul
- Normal 1 hour CCI – 14,700 24 hour CCI – 8000
- Refractory Two consecutive transfusions with 1 hour CCIs less than 5000-7500
- Post transfusion platelet recovery rate <20%
- Normal recovery rate = 65%
- Can also use absolute post count increment

Formulas

TABLE 18-1. Determination of Response to Transfused Platelets

Calculation of Corrected Count Increment (CCI)

\[
CCI = \frac{\text{Body Surface Area (m}^2\text{) \times \text{Platelet Count Increment} \times 10^{11}}}{\text{Number of Platelets Transfused}}
\]

EXAMPLE: If \(4 \times 10^{11}\) platelets are transfused to a patient whose body surface area is 1.8 m\(^2\), and the increase in posttransfusion platelet count is 25,000/\(\mu\)L, then:

\[
CCI = \frac{1.8 \text{ m}^2 \times 25,000/\mu\text{L} \times 10^{11}}{4 \times 10^{11}} = 11,250
\]

Calculation of Posttransfusion Platelet Recovery

\[
\text{PPR(\%)} = \frac{\text{Estimated Total Blood Volume}^* \times \text{Platelet Count Increment} \times 10^2}{\text{Number of Platelets Transfused}}
\]

*Total blood volume can be estimated in adult patients as 75 mL/kg.

EXAMPLE: If \(4 \times 10^{11}\) platelets are transfused to a 70-kg patient and the increase in posttransfusion platelet count is 25,000/\(\mu\)L, then

\[
\text{PPR} = \frac{(70 \text{ kg} \times 75 \text{ mL/kg}) \times 25,000 \text{ platelets/\muL} \times 10^2}{4 \times 10^{11} \text{ platelets}} = 0.328 = 32.8\%
\]

Keys to Remember

• Get the count within 10-60 minutes
• If next day count, use CCI <5000 for refractory
• Likelihood of third failure after two is <50%
• After three, likelihood is 68% failure
• After four, 75% failure

Non-Immune Causes of Refractoriness

- Major cause of refractoriness is non-immune\(^7\)
- May account for up to 88% of refractoriness\(^8\)

<table>
<thead>
<tr>
<th>TABLE 55.1 Etiologies of Platelet Refractoriness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Immune Causes</td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Infection</td>
</tr>
<tr>
<td>Disseminated Intravascular Coagulopathy</td>
</tr>
<tr>
<td>Medications</td>
</tr>
<tr>
<td>Bleeding</td>
</tr>
<tr>
<td>HPC Transplant</td>
</tr>
<tr>
<td>Graft Versus Host Disease</td>
</tr>
<tr>
<td>VOD</td>
</tr>
</tbody>
</table>

HLA = human leukocyte antigens; HPA = human platelet antigens.

CCI and Cause of Refractoriness

- 1 hour CCI – initial recovery
- 18-24 hour CCI – recovery and survival/consumption platelets
- Poor 18 hour – look at 1 hour CCI
- Poor 1 hour – immune refractoriness
- Good 1 hour, poor 18 hour – non-immune destruction\(^5,9\)
- Poor 18 hour – shorter patient median survival related to underlying illnesses
- Three fold increase in bleeding regardless of platelet count\(^10\)

Drug Antibodies and Refractoriness

- Drug dependent platelet reactive antibodies
- In heme-onc, use of vancomycin
- Drug independent antibodies – stimulate antibody formation but not needed later
- Drug dependent antibodies – drug present which binds to epitope
- Any of standard testing platforms can be modified to test
- Make sure proper controls are used

Drugs which Cause Refractoriness

### TABLE 18-5. Drugs Confirmed to Elicit Drug-Dependent Platelet-Reactive Antibodies In Vitro Using Flow Cytometry Testing

<table>
<thead>
<tr>
<th>Drug Category</th>
<th>Individual Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitor</td>
<td>Lisinopril</td>
</tr>
<tr>
<td>Analgesic</td>
<td>Acetaminophen,* propoxyphene</td>
</tr>
<tr>
<td>Antibiotic, antifungal</td>
<td>Sulfamethoxazole, vancomycin</td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone, levofloxacin, nafcillin, piperacillin, rifampin, trimethoprim</td>
</tr>
<tr>
<td></td>
<td>Ampicillin, amoxicillin, cefaltolin, cefadroxil, cefetame, cefpodoxime, cefazidine, cefotaxime, cefpodoxime, ciprofloxacine, ethambutol, linezolid, loracarbef, metronidazole, nitrofurantoin, sulfisoxazole</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td></td>
<td>Phenytoin</td>
</tr>
<tr>
<td></td>
<td>Lorazepam, valproic acid</td>
</tr>
<tr>
<td>Antidepressant, antipsychotic</td>
<td>Amitriptyline, bupropion, haldol, olanzepine, sertraline</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>Atenolol, propranolol</td>
</tr>
<tr>
<td>Cardiac</td>
<td>Amiodarone</td>
</tr>
<tr>
<td></td>
<td>Dobutamine</td>
</tr>
<tr>
<td>Chemotherapeutic agent</td>
<td>Oxaliplatin</td>
</tr>
<tr>
<td></td>
<td>Gelantamycin, irinotecan, suramin</td>
</tr>
<tr>
<td>Cinchona alkaloid</td>
<td>Quinine, quinidine</td>
</tr>
<tr>
<td>Diuretic</td>
<td>Furosemide</td>
</tr>
<tr>
<td>GPIIb/IIIa inhibitor</td>
<td>Abciximab, eptifibatide, tirofiban</td>
</tr>
<tr>
<td></td>
<td>Orbifiban, xemolfilbin</td>
</tr>
<tr>
<td>Histamine receptor antagonist</td>
<td>Fexofenadine, ranitidine</td>
</tr>
<tr>
<td>Narcotic</td>
<td>Fentanyl</td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory</td>
<td>Naproxen*</td>
</tr>
<tr>
<td></td>
<td>Celecoxib, ibuprofen, oxaprozin</td>
</tr>
<tr>
<td>Proton pump inhibitor</td>
<td>Esomeprazole, lansoprazole, pantoprazole</td>
</tr>
<tr>
<td>Thrombin inhibitor</td>
<td>Argatroban</td>
</tr>
<tr>
<td>Vasodilator</td>
<td>Papaverine</td>
</tr>
</tbody>
</table>

*Drug-dependent antibodies that were specific for drug metabolites only. ACE = angiotensin-converting enzyme; GP = glycoprotein.

Climbing the Pyramid
Methods for the selection of platelet products for alloimmune-refractory patients

Transfusion Volume 55, Issue 2, pages 235-244, 13 NOV 2014 DOI: 10.1111/trf.12921
MGH Dzik Algorithm

Fig. 1. Decision algorithm for HLA-selected platelets used at Massachusetts General Hospital, Boston.

Vassallo Algorithm

To Do’s

- Test patient for HLA antibodies with specificities for those antibodies
- Get patient HLA typed if not previously done
- Will take days so don’t get impatient
- Antibody determinations –
  - Serological cell based
  - Solid phase – ELISA, microsphere or flow cytometry
### Cell Based Cytotoxic Method

<table>
<thead>
<tr>
<th>NIH method</th>
<th>Positive reaction</th>
<th>Negative reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA antibody + lymphocyte (antigen-antibody reaction)</td>
<td><img src="image1" alt="Positive reaction" /></td>
<td><img src="image2" alt="Negative reaction" /></td>
</tr>
<tr>
<td>Complement dependent cytolysis</td>
<td><img src="image3" alt="Positive reaction" /></td>
<td><img src="image4" alt="Negative reaction" /></td>
</tr>
<tr>
<td>Cellular staining + fixation</td>
<td><img src="image5" alt="Positive reaction" /></td>
<td><img src="image6" alt="Negative reaction" /></td>
</tr>
</tbody>
</table>
Cell Based Cytotoxic Method

• Complement dependent cytotoxicity

• Lymphocytes in microplate well, add:
  – Patient antibodies
  – Anti human globulin
  – Complement

• Patient antibody binds to cells with corresponding antigen, binds AHG and complement, lyses cell, dye enters cell

• Only for HLA, not HPA or ABO

• PRA score is percentage of cells positive

• PRA scores of 20% or more in alloimmunized refractory patients

Solid Phase HLA Antibody Assays

**CELL-BASED ASSAYS**
- CDC cross-match
- Flow cytometry cross-match

**SOLID-PHASE ASSAYS**
- ELISA
- Luminex

<table>
<thead>
<tr>
<th>Cell-based assays</th>
<th>Solid-phase assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>• CDC-XM reduced the incidence of hyperacute rejection</td>
<td></td>
</tr>
<tr>
<td>• Inability to identify the antigen causing positive</td>
<td></td>
</tr>
<tr>
<td>• Sensitive with high degree of specificity to donor antigens, luminex more sensitive than ELISA</td>
<td></td>
</tr>
<tr>
<td>• Capable of quantifying anti-HLA antibodies level</td>
<td></td>
</tr>
</tbody>
</table>
Solid Phase HLA Antibody Testing

- Known solubilized HLA and HPA antigens bound to solid matrix, beads or microplate
- Add patient antibodies which bind to antigens
- Add labeled AHG, enzyme for ELISA or florescent for flow or Luminex to create a signal which can then be measured
- ELISA more sensitive than CDC but less than flow
- Estimate of incompatibility by cPRA % so 60% means incompatible with 60% of population
- Newer variation use C1Q instead of AHG antibodies to detect antibodies which bind complement
How Much is Enough?

- ELISA and Flow – good detection of antibodies
- Do they cause refractoriness?
- May not cause refractoriness unless detected by CDC
- May lead lab to increase the cutoff for positive antibody to dial down sensitivity

Back to the Bedside – First Steps

• Use ABO identical platelets
  – ABO mismatch can cause poor increment\(^{21}\)
  – Review of 19 studies, platelet count increment consistently higher with ABO identical\(^{22}\)

• Use fresh platelets <=3 days from collection
  – Older platelets reduce CCI\(^{23}\)

• Give at least 2-3 transfusions of these types to determine if the low CCI persists

• Remember to irradiate to prevent TA-GVHD

• 4% of heme-onc patients will require matched products\(^{24}\)

Next Step Crossmatch Platelets

- Solid phase test using rbc adherence, ELISA or flow
- Can be used for HLA class 1, ABH or PLT antibodies
- SPCRA –
  - take donor platelet samples to bind to well
  - Add patient serum with antibodies
  - Add indicator rbcs coated in AHG
  - Spin and read
  - RBC button on bottom is negative
  - Dispersed rbc is positive for antibody to platelets

Grading Chart

<table>
<thead>
<tr>
<th>Hemagglutination</th>
<th>Capture®</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td>1+</td>
</tr>
<tr>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>1+</td>
<td>3+</td>
</tr>
<tr>
<td>0</td>
<td>4+</td>
</tr>
</tbody>
</table>
ELISA and Flow

• ELISA –
  – Donor platelets incubate with mouse anti platelet antibody
  – Micro-well coated with goat anti mouse antibodies to bind donor platelets
  – Patient serum has platelet antibodies
  – Enzyme labeled goat anti-human antibodies to convert substrate and create signal

• Flow – patient serum incubated with donor platelets, add fluorescent tagged antihuman IgG antibodies
Pros and Cons of Platelet Crossmatch

• Most of US uses SPCRA
  – Can find platelets but antibodies may be to antigen not on platelets, so compatible but there may be undetected antibodies
  – Speed to transfusion, NY has 30-50 platelets available to crossmatch against, available in few hours

• Can provide ABO, HLA and HPA compatible platelets

• Best for low to moderate sensitization, not highly sensitized patients
• Progressive alloimmunization did not occur to mismatched antigens in crossmatched patients

• cPRA >60% - difficult to find donors

• Meta analysis 26 papers – 6 studies showed better CCI with cross match compared to non-crossmatch or crossmatch incompatible

• Success rate 50-90% dependent on non-immune refractoriness

• So – use crossmatch platelets while waiting for HLA antibody or antigen typing studies or HLA antibody negative

HLA Antibody Positive, Now What?

- HLA antibody positive- Class 1 HLA-A or HLA-B\textsuperscript{27}
- Japan – may see HLA-C antibodies\textsuperscript{28}
- Do you always get them?
- 1200 intensively transfused patients:
  - 2% HPA, 3% HPA and HLA, 25% HLA antibodies only\textsuperscript{29}
- Takes 2 – 5 weeks to form, 4 days to re-activate\textsuperscript{30,31}
- 15-40% patients increase cPRA score\textsuperscript{30}
- Median persistence 14 weeks, 70% disappear in 1 year\textsuperscript{30}
- Persistence seen in previously pregnant women, and high PRA patients
- If antibody disappears – 50% chance it doesn’t reappear if challenged\textsuperscript{30}

\textsuperscript{31} Howard, JE et al. Transfusion. 1978, Vol. 18, pp. 496-503.
Antigen Negative Platelets & Pool Size

• Ag negative platelets
  – Similar to rbc ag neg.
  – Use platelets negative for identified antibodies

• Pool size

• 7247 HLA typed donors for 29 patients
  – 6 A matches, 33 BU matches
  – 1426 antigen negative match

• More likely to have available products quickly

• Can be used to support moderate and highly sensitized patients

Pool Size for HLA Match by Compatibility

- Brazil 65,500 HLA typed bone marrow donors
- For 154 HLA typed patients
- Heterogeneous population – African, EuroCaucasian, Amerindian
- Mathematical modeling

- Pool size for 80% of patients to have five donors
  - 31940 – complete compatible (A, B1U, B2U)
  - 1710 – possible compatible (B1X, B2UX, 1CREG)
  - 321 – less compatible (B2X, 2 CREGs)

- Pool size for 100% to have five donors
  - NA - complete compatible (A, B1U, B2U)
  - 23,393 - possible compatible (B1X, B2UX, 1CREG)
  - 2500 - less compatible (B2X, 2 CREGs)

Antigen Negative vs. HLA Match Platelets

• Post transfusion Recovery Rates\textsuperscript{32}

• Mean adjusted PPR equivalent among all groups except random
  – HLA – A and BU match – 20.77%
  – Crossmatch 23.38%
  – Antigen negative 24.13%
  – Random 14.87%

• Remember to test monthly for new antibodies

Highly Sensitized Patients and Ag Neg Platelets

• Does every antibody matter? Can you discriminate and eliminate least important?

• Use complement fixing test for HLA antibodies

• Retrospective testing in highly sensitized individuals receiving crossmatch incompatible units

• Original cPRA – 94%, complement screen cPRA – 60%

• CCI higher in complement compatible crossmatch units than incompatible – 10.6 vs 2.5

• Antibodies to HLA-B44 and B45 can be ignored as platelets express weakly

• Similarly, Bw4 and Bw6 on HLA-B13 & B-14 platelets

Next Step – At the Top of the Pyramid

- Patient incompatible, CCI’s low for crossmatch and antigen negative platelets – HLA match next stop
- Donor availability is the issue
- Modeling shows one patient requires pool of 1000-3000 HLA typed donors
- Even with pool this size, only 10% patients receive only HLA matched platelets

- Donor is not a cow for milking
- Blood centers revolve around our donors’ schedules
- 3 day turnaround between request and release

Matching

80% antibodies are directed at public epitopes\textsuperscript{38,39}

Two matching schemes – Duquesnoy\textsuperscript{40}

Classic

- Mismatched antigens which share public epitopes less likely to find antibodies
- HLA-A divided into 3 cross reactive groups (CREGS)\textsuperscript{1}
- HLA-B divided into 4 CREGS\textsuperscript{1}
- A, B1U, B2U are best matches – all others are incompatible which may lead to more antibodies

Know your match – 1 study – 43% “HLA match” were poor B or C matches\textsuperscript{41}

No A or B donors – C match works 73% of time in patients with PRA <60%\textsuperscript{42}

\begin{tabular}{|c|c|c|c|c|}
\hline
Grade & Description & R/D & HLA typing \\
\hline
A & HLA identical – all 4 antigens & R & A1 A2 B7 B8 \\
BU & Only 3 antigens detected – all identical & D & A1 A2 B7 B8 \\
B2U & Only 2 antigens detected – both identical & D & A1 – B8 – \\
BX & 4 antigens detected – 3 antigens identical and 1 cross-reactive & D & A1 A24 B7 B8 \\
BUX & 3 antigens detected – 2 identical and 1 cross-reactive & D & A1 A24 – B8 \\
B2X & 4 antigens detected – 2 antigens identical and 2 cross-reactive & D & A1 A24 B7 B64 \\
C & 1 antigen mismatch, out-of-CREG & D & A1 A32 B7 B8 \\
D & All other ≥2 antigen mismatches & D & A1 A32 B7 B64 \\
\hline
\end{tabular}

R: recipient; D: donor

Later version – Duquesnoy

Assumes immunogenic epitopes represented by triplet amino acid sequences which characterize it

Newest version – “eplets” – amino acid sequences which characterize protein antigen antibody binding complexes

Eplet - all AA within 3 Angstrom of antibody binding position – accounts for folding and 3-D structure

Donor triplets or eplets will not be immunogenic if patient has them regardless of HLA typing – functionally identical

Software calculates number of non-shared epitopes between donor and patient to come up with best matches

Paper Battle

• Limitations
  – HLA – match grade
  – Crossmatch & antigen negative – PRA%

• Perfect match A or B1U – better 24 hour recovery but similar 1 hour recovery to crossmatched platelets\(^{45}\)

• Less than perfect match then crossmatch is equivalent\(^{46}\)

• Antigen negative
  – PRA <70% then 80% successful transfusion
  – PRA >70% then 25% successful transfusion

• Crossmatch
  – PRA < 80% - did well
  – PRA > 80% - many failures due to missing antibodies\(^{47}\)

• Small randomized study 9 patients, 142 platelet transfusions

• If No A or Bu match – randomized to CREG matching or epitope based matching (HLAMATCHMAKER)

• A/BU match 85% success CCI>7500

• CREG match 63%, Epitope match 83%48

• Recent retrospective review 32 patients 354 transfusions

• 25% crossmatch, 30% HLA match, and 12% random 1 hour CCI >5000

• Perspective: role of matching limited by non-immune refractoriness

• Recommend limited use (2) of crossmatch and/or HLA, if unsuccessful go back to random apheresis49

Human Platelet Antigens

• 7-8% of alloimmunized patients have HPA antibodies\textsuperscript{50}
• Five antibody causing antigens – GP1a, GP1b, GP2b, GP3a, CD109
• Test for HPA antibodies through solid phase ab screen
• Most common HPA-1b and HPA-5b, not as bad as HLA abs\textsuperscript{29,51}
  – Antigen frequency < 30%
  – Autoreactive antibodies not as pathogenic as HLA abs
  – Low expression of some antigens on platelets
• Small pool of GP1a negative donors or platelet crossmatching

What’s Next?

- No HLA or HPA antibodies present – non-immune
- Previous good response or no response to excellent match – look for both immune and non-immune causes
- Block immune mediated destruction – IVIg results are variable
- Suppress antibody production – Vincristine, cyclosporine, staph A columns, plasma exchange – limited success
- Rituxan weekly or 1 dose with plasma exchange & IVIg
- Modified platelets – under investigation
- IL-11 – licensed to prevent thrombocytopenia in myelosuppressive but not myeloablative chemotherapy

More Next Steps – Farther Down the Road

- Abatacept – modulates T-cell costimulatory signaling to block refractoriness and HLA alloimmunization in MICE\textsuperscript{57}

- Strip platelet of HLA – cold citric acid
  - 70-90\% HLA removed
  - Viability and aggregation maintained
  - Protect against HLA complement lysis and monocyte mediated phagocytosis\textsuperscript{59}

\textsuperscript{57} Waterman HR et al. Transfusion 2016 Vol. 56 pp. 91-100 \textsuperscript{59} Meinke S et al. Transfusion 2016 Vol. 56 pp. 370-82
Prevention

- Limit donor exposure by using apheresis platelets rather than whole blood platelets\textsuperscript{54}
- Use leukoreduced platelets – meta-analysis 8 randomized controlled trials – leukoreduction can reduce alloimmunization and refractoriness\textsuperscript{55}
- UVB irradiation – not licensed in US\textsuperscript{50}
  - Prevents donor dendritic cell interaction with patient T-cell
- Mirasol – Riboflavin & UVB – prevent HLA immune stimulation in mice\textsuperscript{58}
- Limit platelet use by following transfusion trigger – 10,000 in non-bleeding patient
- 13 or more apheresis units found to be a cause of alloimmunization\textsuperscript{24}

\textsuperscript{58} Jackman RP et al. Transfusion 2013 Vol. 53. pp.2697-709
I Reached the Top and This is All I Saw!
Things to Do

• Suboptimal 18 hr CCI – look at 1 hr CCI
  – More antibodies?
  – Non-immune cause?

• Expand pool of available donors
  – Remove CMV negative requirement as apheresis are leukoreduced already – CMV negative equivalent
  – Go for HLA matched ABO incompatible donors
    • ABO incompatible not as bad as HLA incompatible\(^56\)

• Treat the right thing, platelet count vs. bleeding
  – Hypofibrinogenemia, coagulation factor defects, low vWF – all treated with transfusion
  – Use of Amicar for mucosal or serosal bleeding

TABLE 1. Pitfalls in HLA-selected PLT support that require communication to prevent

- Once a high PRA is established, the clinical team continues to request futile unmatched PLTs on a daily basis and the laboratory fills these requests. This may only serve to boost antibody titers in the recipient!
- The laboratory fails to pursue the rationale behind repeated requests for HLA-selected PLTs, which upon investigation would prove not to be a valid indication for PLT transfusion in the first place.
- The clinical team fails to obtain HLA typing on the patient and requests HLA-matched PLTs at a time when the patient has no circulating lymphocytes available to type. (Fortunately, this pitfall can be overcome by HLA labs that use DNA-based typing techniques.)
- The laboratory fails to establish “new expectations” with the clinical service (often the clinical residents and nurses) regarding what is the appropriate new target PLT count, indication for PLTs, or expected frequency of transfusions for the alloimmunized patient.
- The laboratory fails to alert the floor once the HLA-selected PLTs arrive at the hospital and, as a result, the PLTs outdate.
- The requesting service is too inflexible and will not transfuse HLA-selected PLTs that have arrived at the hospital. The PLTs outdate on the rotator. A few hours later, the service requests PLTs.
- The laboratory is so restrictive in its approach to HLA selection that by the time PLTs finally do arrive at the hospital (days after the initial request), the patient no longer needs them or has been discharged.
- The laboratory fails to inform their PLT supplier (regional blood center) that special PLT support is no longer needed and, as a result, HLA-selected PLTs are acquired when they are no longer needed.