Platelet Refractoriness: The Basics

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Objectives

• Define platelet refractoriness and associated conditions that may cause platelet refractoriness.
• Describe how platelet refractoriness may be diagnosed.
• Describe technical methods that may be used to provide information to help manage refractory patients.
Definitions

Platelet refractoriness:
A patient is refractory to platelet transfusions if the patient’s circulating platelet levels consistently fail to increase by at least 10k/µliter after transfusion of an appropriate dose of platelets.
Clinical implications

- Platelet refractoriness connotes a worse survival
- Increased exposure to platelet concentrates
- Increased time spent at critically low platelet concentrations
- Increased bleeding complications
- Most common in chemotherapy and BMT pts

Kerkhoffs et al. 2008
Toor et al. 2000
Definitions

Immune-mediated platelet refractoriness:
Immune-mediated refractoriness is due to antibodies made by the patient that recognize an epitope on the transfused platelets, most commonly human leukocyte antigen (HLA) class I.
Definitions

Non-immune-mediated platelet refractoriness:

Non-immune-mediated refractoriness is due to a process other than platelet allo-antibodies which significantly decreases the circulation time of transfused platelets.
Definitions

Non-immune-mediated platelet refractoriness:
Non-immune causes include splenomegaly, diffuse intravascular coagulopathy (DIC), fever, infection (sepsis), ongoing bleeding, graft-versus host disease, veno-occlusive disease, and many medications.
Immune refractoriness

- Alloantibodies produced by the patient recognizing antigens on the transfused platelets
  - Human Leukocyte Antigen (HLA) class I antigens
  - Human platelet antigens (HPA)
  - ABO antigens

- Antibodies bound to platelets target the platelets for removal in the reticuloendothelial system
Human Leukocyte Antigens

- HLA proteins are essential components of immune system surveillance.
- HLA-II expressed on APC to present antigens from outside the cell to monitor for bacterial/fungal/etc infections.
- HLA-I proteins expressed on most cells to present internal antigens to help monitor for cancer and viral infection.
Human Leukocyte Antigens

- HLA genes on each chromosome 6p
- Thousands of different alleles for each locus (A, B, C)
- Patient can recognize any foreign antigen and form antibodies against that antigen
- Shared antigenic epitopes (public epitopes) can result in reactivity to multiple HLA phenotypes

<table>
<thead>
<tr>
<th>MHC class I</th>
<th>locus</th>
<th>#</th>
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<tr>
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<td>HLA A</td>
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</tr>
<tr>
<td></td>
<td>HLA C</td>
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</table>
Human Platelet Antigens

- Epitopes on glycoprotein complexes expressed on the platelet cell membrane
- Human Platelet Alloantigens (HPA) 1-15
- Antigens to which patients have developed antibodies
- As with other antigens, patients may develop antibodies to antigens which they lack
Human Platelet Antigens

- Development of anti-HPA antibodies cause:
  - Post-Transfusion Purpura
  - Neonatal Allo-Immune Thrombocytopenia
  - Post-Transfusion Platelet Refractoriness

- HPA typing is done by sequence-specific PCR

- HPA antibodies identified using antibody sandwich

Metcalfe P. 2004. Vox Sanguinis
ABO Antigens

- Inherited by presence of enzyme that makes A or B from H substance
- Inheritance of 1 copy (chromosome 9) sufficient for A or B expression
- Similar CHO chains present on surface of gut bacteria
- ABO is expressed at low levels on platelet membrane
- In Le(b+) individuals (so Se+, Le+ or FUT2+, FUT3+), soluble A/B is passively adsorbed to platelet surface
Definitions

Pooled platelets (5-pack):
- Preparation of platelets made from the platelet fraction of the whole blood donations from 5 separate donors.
- Total of at least $3 \times 10^{11}$ platelets which should increase circulating platelet concentration by 30-50 K/μL

Single donor platelets (apheresis):
- Platelets from a single donor (collected by pheresis) with the same number of platelets as a pooled platelet unit.
- Total of at least $3 \times 10^{11}$ platelets which should increase circulating platelet concentration by 30-50 K/μL
Definitions

Cross-matched platelets:
Single donor platelets (by apheresis) which are evaluated with the patient’s serum for compatibility.

HLA antigen-negative platelets (HLA matched):
Single donor platelets which are collected from a patient whose HLA class I phenotype is compatible with the patient’s HLA antibody panel.
Evaluation requested by clinician

≥ 3 platelet transfusions with 1-hr post-transfusion counts  < 3 platelet transfusions with 1-hr post-transfusion counts
Circulating platelets vs. Time
Circulating platelets

Time

Circulating platelets

Time
Circulating platelets

Time

- Immune-mediated
- Splenic sequestration
- DIC

Circulating platelets

Time
Evaluation requested by clinician

≥ 3 platelet transfusions with 1-hr post-transfusion counts

< 3 platelet transfusions with 1-hr post-transfusion counts

Calculate CCI

CCI at 1 hr low

CCI at 1 hr high

Cannot determine
CCI

- Corrected count increment – calculation to evaluate platelet increase increment
- Corrects for recipient size and platelet unit dosage

\[
CCI = (\text{post-plt} - \text{pre-plt}) \times \text{BSA}^2
\]

Dose of platelets

CCI of < 7 is generally considered a poor response, suggesting platelet refractoriness
Evaluation requested by clinician

≥ 3 platelet transfusions with 1-hr post-transfusion counts

Calculate CCI

CCI at 1 hr low
- 2 tubes for CXM

High compatibility

CCI at 1 hr high
- Not platelet refractory

< 3 platelet transfusions with 1-hr post-transfusion counts

Cannot determine

Low compatibility
Platelet cross-match

Patient plasma is added to immobilized aliquots of single-donor platelet units

Binding of indicator RBCs shows presence of antibodies recognizing antigens on the platelets

# compatible/total # tested suggests level of immune-mediated refractoriness

Donor

Recipient

Positive

Negative
Evaluation requested by clinician

≥ 3 platelet transfusions with 1-hr post-transfusion counts

Calculate CCI

CCI at 1 hr low

2 tubes for CXM

High compatibility

Use CXM platelets

Low compatibility

HLA and HLA-PRA testing

HLA-PRA low

HLA-PRA high

CCI at 1 hr high

Not platelet refractory

< 3 platelet transfusions with 1-hr post-transfusion counts

Cannot determine
HLA-PRA and HLA typing

- Most common target of antibodies in immune-mediated platelet refractoriness
- HLA-PRA tested for via flow cytometry using beads coated with purified HLA antigens
- Quantifies sensitization and gives Ab specificity
- Patient’s HLA type determined by sequencing
HLA matched platelets

- HLA phenotype is combination of 2 haplotypes
- Any mismatches which introduce Ag not present in the recipient can result in Ab production
- Haploidentical donors expand the potential pool
- Even “matched” platelets may not be 6/6 match
Evaluation requested by clinician

≥ 3 platelet transfusions with 1-hr post-transfusion counts
- Calculate CCI
- CCI at 1 hr low
  - 2 tubes for CXM
    - High compatibility: Use CXM platelets
    - Low compatibility: HLA and HLA-PRA testing
      - HLA-PRA low: Use CXM platelets
      - HLA-PRA high: Use HLA-matched platelets

< 3 platelet transfusions with 1-hr post-transfusion counts
- Cannot determine

Evaluation requested by clinician

≥ 3 platelet transfusions with 1-hr post-transfusion counts

Calculate CCI

CCI at 1 hr low

Use CXM platelets

High compatibility

HLA-PRA low

Use CXM platelets

HLA-PRA high

Use HLA-matched platelets

CCI at 1 hr high

Not platelet refractory

HLA and HLA-PRA testing

HLA-PRA low

Use CXM platelets

HLA-PRA high

Use HLA-matched platelets

< 3 platelet transfusions with 1-hr post-transfusion counts

Cannot determine
Evaluation requested by clinician

≥ 3 platelet transfusions with 1-hr post-transfusion counts

Calculate CCI

CCI at 1 hr low

HLA and HLA-PRA testing

HLA-PRA low

Random platelets

< 3 platelet transfusions with 1-hr post-transfusion counts

CCI at 1 hr high

Not platelet refractory

HLA-PRA high

Use HLA-matched platelets

Cannot determine
Platelet availability

- CXM platelets are NOT currently available in this region
  - If they were available, still NOT available on emergent basis
  - Testing usually results in >2 business day availability
- HLA-matched platelets are NOT available on an emergent basis
  - Testing, identification of a donor, collection of platelets, and transportation usually results in >7 day availability
- For emergent use, only pooled platelets/ unmatched apheresis platelets are available
Non-immune platelet refractoriness

Increased consumption or activation

- On-going bleeding
- DIC
- Infection
- TTP
- Vasculopathy

Must treat underlying disease while maintaining vascular stability
Non-immune platelet refractoriness

Sequestration/destruction

- Splenomegaly
- ITP
- Autoantibodies which recognize platelet epitope - most commonly GPIIb/IIIa: Glanzmann’s
- May also be secondary to drugs affecting Ag-Ab interaction
- Treat underlying disease and mitigate platelet destruction

Non-immune platelet refractoriness

Decreased production
- Chemotherapy
- Leukemia
- Marrow infiltration

Physiologic use of 7-8k platelets daily
- At normal levels ~ 10%
- At low levels ~ 90%

Mueller-Eckhardt et al, Br J Hematology 1982;52:49-58
Management of refractory patient

If immune-mediated:

- For prophylactic transfusions – consult with HLA lab, Blood Bank, and blood supplier to trial HLA or cross-matched platelets

- For acute bleeding – transfuse with standard platelet units and utilize other techniques to minimize bleeding
Management of refractory patient

If non-immune-mediated:

➢ For prophylactic transfusions – recognize that a specific goal (platelet = ##) may not be attainable

Risk of clinically significant spontaneous bleeding is only increased with PLT < 5k

Schlicter et al, NEJM 2010;362:600-613
Management of refractory patient

If non-immune-mediated:

- For prophylactic transfusions – recognize that a specific goal (platelet = ##) may not be attainable
- Transfuse to minimize spontaneous bleeding and to treat on-going bleeding
- For procedure or surgery - ?
Management of refractory patient

If non-immune-mediated:

- For prophylactic transfusions – recognize that a specific goal (platelet = ##) may not be attainable
- Transfuse to minimize spontaneous bleeding and to treat on-going bleeding
- For procedure or surgery – transfuse during procedure/surgery to maximize benefit for patient from transfusion
Questions?

1. Platelet party!

2. What's going on here?
   Platelet party!

3. Why, I haven't had this much fun in years!

4. I think we're stuck.
   Platelet party.
   No, seriously, we're stuck.
   Platelet party!

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