Liquid Gold: Platelet Testing

Ralph R. Vassallo, MD, FACP
Vitalant Chief Medical & Scientific Officer
Disclosures & Goal

Disclosures

Advisory Boards
- bioMérieux
- Fresenius-Kabi
- Hemanext
- HemaStrat
- Roche Molecular

Other Activity
- Cerus Corp. (DSMB)
- Immucor (Speaker)

My Goals

- Review shared and unique antigens present on platelets
- Discuss antigen typing & antibody screening / identification
- Touch on disease-specific testing and management strategies
Platelet Surface Antigens

• ABH Antigens
  • Expressed on major glycoproteins
  • Major mismatch can decrease post-transfusion yield
    • $A_2$ platelets have little to no A Ag
• HLA Class I (A & B, very little C)
  • Inherited as maternal / paternal haplotypes
  • 30 Caucasian haplotypes represent ~75% of those seen
• HPAs
Human Platelet Alloantigens (HPAs)

“Fibrinogen receptor”
50,000 – 80,000 surface copies

“vWF receptor”
25,000 GP1b surface copies

“Collagen receptor”
2,800 – 8,000 surface copies

Cell-cell-substrate
~2,000 surface copies

Higher freq. Ag designated ‘a’, lower ‘b’; Ags to which Ab discovered only to the low-freq. / non-wild type Ag labeled ‘bw’.
Platelet Isoantigen

- An iso-Ab to CD36 (Platelet GP IV; ‘Nak\(^a\) Ag’; a class B scavenger receptor protein), may be formed by individuals of Asian, African and Palestinian descent who lack CD36 on all cells (platelets, monocytes, endothelium, some tissue epithelia)

- 2-14K copy platelet surface density
  - Most “deficient” individuals lack CD36 only on platelets

- 1-10% Asians and <8% Africans lack CD36 on platelet testing (and can serve as Ag-neg platelet donors); only 10-40% of these lack CD36 on all cells and can form Nak\(^a\) Ab
HPAs

- 6 biallelic systems with Caucasian / African frequency > 1% (except 4b)
- Another 23 (and growing) low-frequency ‘bw’ antigens (up to HPA-29bw at present)
- All single-nucleotide polymorphisms except for HPA-14bw which has a triplet deletion

<table>
<thead>
<tr>
<th>HPA Nomenclature</th>
<th>Caucasian Phenotypic Frequency</th>
<th>African Phenotypic Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-1a</td>
<td>97%</td>
<td>99%</td>
</tr>
<tr>
<td>HPA-1b</td>
<td>31%</td>
<td>18%</td>
</tr>
<tr>
<td>HPA-2a</td>
<td>99%</td>
<td>96%</td>
</tr>
<tr>
<td>HPA-2b</td>
<td>23%</td>
<td>34%</td>
</tr>
<tr>
<td>HPA-3a</td>
<td>87%</td>
<td>89%</td>
</tr>
<tr>
<td>HPA-3b</td>
<td>59%</td>
<td>56%</td>
</tr>
<tr>
<td>HPA-4a</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>HPA-5a</td>
<td>99%</td>
<td>98%</td>
</tr>
<tr>
<td>HPA-5b</td>
<td>21%</td>
<td>23%</td>
</tr>
<tr>
<td>HPA-15a</td>
<td>70%</td>
<td>91%</td>
</tr>
<tr>
<td>HPA-15b</td>
<td>79%</td>
<td>51%</td>
</tr>
</tbody>
</table>
HPA / CD36 Antibodies

• Responsible for several clinical syndromes
  - Fetal / Neonatal Alloimmune Thrombocytopenia (FNAIT)
  - Platelet Transfusion Refractoriness
  - Post-Transfusion Purpura (PTP)
  - Passive Alloimmune Thrombocytopenia
  - Post-HSCT Alloimmune Thrombocytopenia
  - Immune Thrombocytopenic Purpura (ITP)

  • Primary or Secondary (HIV/HCV, lymphoproliferative disorders, Systemic Lupus Erythematosus, Common Variable Immunodeficiency, post-Hematopoietic Stem Cell Transplant)
Whole Platelet Assays
(HPA assays complicated by HLA and ABO antibodies)

✓ PIFT
✓ Mixed Passive Hemagglutination (SPRCA)
Platelet Immunofluorescence Test (PIFT)

Direct test using known antisera (Patient antigen [Ag] typing)
Indirect test using typed platelets (Serum antibody [Ab] detection)
Solid Phase Red Cell Adherence (SPRCA)

Platelet Monolayer

Reactive / Incompatible

Non-Reactive / Compatible

Anti-Platelet Ab
Indicator Red Cell
Coated with Anti-Human IgG

Used for Ag typing, Ab detection and platelet crossmatching
Glycoprotein-Specific Assays

✓ ELISA
✓ MACE
✓ MAIPA
✓ Bead-Based Detection
ELISA (ACE), **Modified Ag Capture ELISA & Monoclonal Ab Immobilization of Platelet Ags**

![Diagram showing ELISA procedure](image)

- **Patient Serum**
  - Platelet
  - Murine GP-specific MoAb
  - Incubate, wash, lyse

- **Enzyme-labeled anti-human IgG**
  - Patient GP-specific IgG Ab
  - Platelet GP
  - Murine GP-specific MoAb

- **ACE**
  - Anti-mouse IgG

- **MACE**

- **MAIPA**
Bead-Based Antibody Detection

Patient serum with HPA-specific Ab

PE-conjugated anti-human IgG

Wash beads

GP Ia/IIa
Molecular Techniques for HPA and HLA Typing

• Sequence-Specific Priming PCR
• Sequence-Specific Oligonucleotide Probes
  • Luminex
  • Other solid-phase arrays
• Real-Time PCR
• Sequence-Based Typing
  • Sanger
  • NextGen

Detect some/all of HPA 1-11 & -15; some platforms can type HPA 1-29
Low-intermediate resolution (2- & partial 4-digit) HLA-A / -B
High resolution (4+-digit) HLA-A / -B
Common HLA Ab Screening Methods

Enzyme-Linked ImmunoSorbent Assay (ELISA)

Luminex Flow Analyzer
- Mixed Beads (screening)
- Single Antigen Beads (ID)
  (30-31 HLA-A / 46-47 HLA-B Beads)

Uses PE-conj. anti-human IgG to detect Ab binding to beads, OR
- C1q & C3d versions to determine C’ binding

Considerations:
- MFI cutoffs 1000-5000; MFI ≠ titer; hook effects;
- C’ binding can be predicted from MFI
FNAIT – Fetal / Neonatal Alloimmune Thrombocytopenia
FNAIT – Mechanisms of Alloimmunization

• By 36 weeks, all women have detectable fetal cells in circulation, which can persist for decades
  - Up to 6% of free DNA in maternal plasma is of fetal origin, clearing within hours of delivery (opportunity for prenatal diagnosis)

• Alloimmunization occurs through fetal hemorrhage or previous (undetected) pregnancies as well as syncytiotrophoblast integrin exposure (GPIIIa)

• HLA-DR restricted antigen presentation
  - e.g., DRB3*01:01 presentation of HPA-1a can be diagnostically exploited

• Suggestion in mouse studies of facilitating role of inflammation
FNAIT Statistics

• 1 per 1,000 to 2,000 live births (1 per 100 infants have platelet count <150 μL due to infection, drugs, maternal ITP, congenital thrombocytopenias, vasculopathies, or FNAIT)

• Most common cause of severe thrombocytopenia and intracranial hemorrhage

• ~40% of cases occur in primiparas

• Diagnosis confirmed by identifying HPA Ab in maternal serum and genotyping parents to confirm incompatibility
  - Positive maternal-paternal crossmatch in high-probability cases may suggest the need for low-frequency paternal antigen typing (anti-HPA-4b, 6bw, 21bw & CD36 in Asians and 9bw in Caucasians)
  - Paternal zygosity useful in managing subsequent pregnancies
FNAIT Implicated Antibodies

- In up to 2/3rds of presumed FNAIT, no Ab is found
- In Caucasians (& Africans):
  - 75-80% due to anti-HPA-1a (some low avidity)
  - 10-15% anti-5b
  - ~5% anti-1b
  - 1-2% anti-3a, -5a, CD36
  - Low frequency paternal antigens implicated in <1% unselected cases (6-7% highly-selected cases)

• Order:
  - Maternal serum – paternal platelet crossmatch
  - Maternal HPA (& sometimes HLA) antibody testing
  - Confirm maternal Ag-negative status
  - May require (directed) genotyping of both parents
FNAIT Treatment

• Unmatched platelets often successful in absence of matched units
• Empiric 1a/5b-negative units, directed Ag-negative donations or washed maternal platelets preferred except in low frequency Ab-mediated NAIT which respond to unmatched platelets
  - Dad is NOT a good donor! 😖
• IVIg helpful in increasing infant counts
• Antenatal management with IVIg ± steroids and/or IUT
Platelet Transfusion Refractoriness
Platelet Transfusion Refractoriness

• “Inappropriately low platelet count increment 24 hours after two consecutive, fresh, ABO-identical transfusions”
  - Alloimmunity primarily in hematologic disease & cancer (3 – 5% of leukemia and transplant patients with universally-leukoreduced products)
    • 24-hr failure in 30 – 50% of products during intensive transfusion; 25 – 70% of these patients have at least one failure
    • Independently associated with greater bleeding and decreased survival
  - 90% of poor 18- to 24-hr counts associated with ≥1 non-immunologic conditions shortening platelet survival:
    • Splenomegaly, fever/infection, DIC, following stem cell transplant / GvHD, sinusoidal obstruction syndrome, amphotericin B use, bleeding
    • 24-hr counts normally 50-65% of 1-hr counts
  - Alloimmunity (HLA >> HPA Ab) confirmed in 30 – 80% of referred sera associated with poor recovery (10- to 120-min counts)
    • Other immune causes (drug-induced Ab; autoAb; circulating immune complexes), massive splenic sequestration / hemorrhage also affect 1-hr recovery
Expected Increments & Refractoriness

• Appropriateness of increment depends upon product content, patient blood volume & splenic pooling

\[ PPR = \frac{\text{Increment} \times 10^9/L}{\text{# Platelets transfused} \times 10^9 / \text{Blood volume} \times 1} \]

Max. obtainable PPR is ~66% due to splenic pooling

\[ CCI = \frac{\text{Increment} / \mu L \times \text{BSA} \times m^2}{\text{# Platelets transfused} \times 10^{11}} \]

Expected 1-hr & 18-24-hr CCIs (in /\mu L/m^2/10^{11} Plts. transfused)
- SDPs: Mean 1-hr – 14,500 and 18-24-hr – 7,750
- RDPs: Mean 1-hr – 12,250 and 18-24-hr – 7,000

• “Refractoriness” defined as 50-60% of expected 1-hr values on 2 consecutive, fresh, ABO-compatible transfusions
  - PPR ≥ 30% ~ 1-hr, ≥ 20% after 18-24-hr (%)
  - CCI ≥ 7,500 ~ 1-hr, ≥ 5,000 after 18-24-hr (/\mu L/m^2/10^{11} plts.)

HLA Alloimmunization

- HLA alloAbs form within 2 – 4 weeks (anamnestic responses seen in 4 – 10 days)

- Majority of HLA Abs (70 – 90%) directed at HLA public epitopes; only a few Abs may cause broad alloimmunization (percent/panel-reactive Ab or PRA)

- Petz et al. observed interesting changes in PRA category assignment (1-9%, 10-79%, 80-99%) in repeat AHG-CDC assays
  - 14% increased, 54% stable and 32% decreased

- Median time to HLA Ab loss in the TRAP trial was 14 weeks (95% CI: 12 – 19 weeks) with ~75% losing Ab by 1 year
  - Due to cessation of antigenic stimuli, tolerance or anti-id Abs

Treatment of Alloimmune Refractoriness

- Predicated on ~85% of alloimmune refractoriness due to HLA-A or -B Ab only, ~10% both HLA & HPA Ab and ~5% HPA Ab only
  - Begins with HLA Class I-based matching (Identical > Ag-negative >> mismatches based upon CREG- or epitope-based guesses); ignores HPA Ab

- Platelet Crossmatching (XM)
  - Detects some significant HPA and most HLA Abs, but also ABO; more widely available, but lower pos. / neg. pred. value; only 475 of 2800 (17%) XMs compatible in one large study – charged by the well, costs can escalate

- Start with HLA matching; with persistent 1-hr failures, suspect HPA Ab
  - Also suspected with negative HLA Ab screen but poor 1-hr recoveries or with XM % reactivity > expected after Ab identification
    - 30% of Abs to HPA-5b (80% of units compatible, i.e., lacking HPA-5b)
    - 20% to HPA-1b (70% compatible)
    - 20% to HPA-15b (20% compatible)
    - 10% to HPA-2b (90% compatible)
      Thus, ~80% due to 4 of 11 high freq. Ags)
HLA Serological CREGs

28 HLA-A (~3,400 proteins) and 62 HLA-B (~4,250 proteins) serologically-determined antigens

Duquesnoy A/BU, BX, C, D matching (with knowledge of statistically poor BX/C matches) & epitope matching with HLAMatchmaker
HPA-Mediated Platelet Refractoriness

• Results only from Abs to higher-frequency Ags (HPAs 1-5, 15 and CD36) in HLA Class II-susceptible alloexposed patients
  - HLA Ab diminished by leukoreduction (LR); HPA Ab unaffected by LR
• Requires critical combination of transfused platelet antigen density, recipient antibody titer and avidity, and recipient reticuloendothelial system appetite for antibody-coated platelets

• Support for significantly HPA-alloimmunized patients requires selection of HPA-negative (and possibly HLA-neg) donors
• XM (of HLA-compatible donors if necessary) best at identifying high Ag density incompatibility
  - IIb/IIla: >50K copies; Ib: ~25K copies; CD36: ~6K copies; Ia/CD109: ~2K copies
• Donor HPA genotyping expensive, but establishes donor pool (HPA genotyping generally covers 1-11 (±10) & 15, missing only CD36)
Management Pearls for Refractory Patients

• 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) Abs
  - Consider brief support if IgM HLA Abs suspected
  - May succeed just because units are fresher & ABO-matched
Post-Transfusion Purpura
Post-Transfusion Purpura

- Thrombocytopenia <20,000 /μL 4-12 days after transfusion
  - 1:25K – 100K platelet-containing transfusions (1:56K Medicare database)
  - Often accompanied by transfusion reaction; 0-13% mortality
  - Lasts 1-10 weeks without Rx; improves in 1-5d, lasts ~2 weeks with Rx
- Attributed to anamnestic HPA alloAb response which broadens to or triggers autoAb (less likely alloAg or immune complex cycling)
  - Usually previously-pregnant females, but seen in alloexposed males
  - Classically (80%) anti-HPA-1a, but also 1b, 3a/b, 4b, 5b, CD36 reported
  - ~85% response to 1g/kg IVIg x2d ± steroids, rescue with PEx for refractory patients (PEx 50-60% response as primary modality)
  - Response to HPA-neg platelets seen after Rx begins
  - Ag-neg platelets, washed RBCs recommended after resolution
Passive Alloimmune Thrombocytopenia

1950: Harrington–Hollingsworth experiment
Passive Alloimmune Thrombocytopenia

• Passive transfusion of HPA antibody
  - Alloimmunized donors (usually female) retain antibody from pregnancy or transfusion for decades
    • Most anti-HPA-1a, but anti-HPA-5b and anti-Nak\textsuperscript{a} reported
    - HPA-1a-negative platelet donors should be screened for antibodies!
    - Usually associated with high-plasma volume products (>85%)
• 1/3\textsuperscript{rd} of recipients have a reaction
  - Fever/chills, hypotension, back pain, respiratory symptoms, rash
• Mean time to single-digit nadir \(~6\) hours; median time to normal count about 5 days
• Bleeding often occurs; controlled with platelet infusion, IVIg ± steroids

Summary

• FNAIT, PTP, Passive Alloimmune and Post-HSCT Thrombocytopenias require HPA Ab/Ag testing to make a definitive diagnosis

• Platelet Transfusion Refractoriness requires HLA Ab/Ag testing and occasionally, HPA Ab/Ag testing; ABH antigens must also be considered for some test methodologies
  - Parsimony in testing can be more costly in the long run

• Common HPA & HLA Ab/Ag testing techniques are available in most reference laboratories, but may at times require more sophisticated testing available only in quite specialized labs
  - (Clinical/genotypic) knowledge of what Abs are suspected helps guide testing
Thank you
Questions?

- You are all muted
- Q&A following session - Type in questions
Questions?

- You are all muted
- Q&A following session - Type in questions
Continuing Education

• PACE, Florida and California DHS
• 1.0 Contact Hours
• Each attendee must register to receive CE at:
  https://www.surveymonkey.com/r/LiquidGoldPlateletTesting
• Registration deadline is 3 January, 2019
• Certificates will be sent via email only to those who have registered 17 January, 2019
Thank you!