American Red Cross HLA Laboratories Playing with Matches

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I have no relevant financial relationship to disclose.

HLA Overview

- HLA = Human Leukocyte Antigen
- HPA = Human Platelet Antigen

Human Leukocyte Antigen System

- HLA Antigens are found on the surface of both platelets and white cells as well as on other nucleated cells of the body.
- Platelets carry Class I HLA antigens and lack Class II antigens.

Human Leukocyte Antigen System (Class I Antigens)

HLA –A, B, C

Found on most body tissue cells

Interact with cytotoxic T cells

Human Leukocyte Antigen System (Class II Antigens)

HLA –DR, DP, DQ

Limited Cellular Expression

Interact with T-helper cells

HLA Antibodies

HLA antibodies can cause the destruction of transfused platelets.

Sensitization may develop due to:

- Pregnancy
- Multiple transfusions

What about HPA?

- Refractoriness can be caused by HPA (platelet glycoprotein) antibodies alone (<6% of patients – although I have seen <1%) or in combination with HLA antibodies (<10%).
  - Some HPA antibodies do not cause recognizable refractoriness due to low pathogenicity and/or low population antigen frequency (OR low titer)….

What about Class II or Cw – they are on the report…

- HLA Class II DR/DQ/DP are mainly of interest for bone marrow or tissue transplants, or disease association (DR).
- HLA Cw is often times reported, but Cw is weakly expressed on platelets, so at this time we don't match for Cw.

Expected Increments

The expected effect under ideal circumstances:

- 30 to 50,000 increase per apheresis platelet

However, the effect depends upon the patient's blood volume and underlying clinical problem:

- Currently known to be consuming platelets?
- Known current cirrhosis and/or hypersplenism?
- Current fever, treatment with amphotericin?

Platelet Refractoriness

Nonimmune factors include:
Fever, infection, hypersplenism, DIC, bleeding, and medications (Amphotericin B; other antibiotics).

Nonimmune factors cause the majority (80%-90%) of platelet-refractory cases.

Immune-mediated factors include:
ABO, Class I HLA,(to a lesser extent) HPA antibodies, drug-dependent platelet antibodies.

Immune factors cause a minority (20%-25%) platelet refractory cases.

Juskewitch JE. *TRANSFUSION* 2017;57;2828–2835

Platelet Refractoriness

A widely accepted definition of refractoriness is when two consecutive platelet transfusions lead to 1 hour post transfusion corrected count increments (CCI) of less than 5000 platelets x m^2 per µL.

We recommend ABO identical.

Considerations

Alloimmunity ≠ refractoriness
(only ~40% with HLA antibodies are clinically refractory)

Presumably though, many of the "non-refractory" transfusions resulted in recoveries <100% predicted.

Just because you have HLA antibodies does not mean you'll exhibit refractoriness.

~ 75% of pts. undergoing leukemia induction lose HLA antibodies within a year, even with ongoing transfusion.

Parous females and those with broad alloimmunity (high PRA (% reactive antibody)) are more likely to have antibody persistence.

Fontaine MJ. *Transfusion* 2011;51:2611-8.

Approaches for investigation of immune platelet refractoriness

- Rule out non-immune causes of refractoriness
- Several 1 hour post transfusion corrected count increments to assess response
- Perform testing for antibodies that may cause alloimmune refractoriness

→ HLA antibody screening/identification
Platelet antibody screening/identification
Platelet Crossmatch

Testing Performed by the PNW ARC HLA Laboratory

- The PNW HLA Laboratory recommends ordering a "New Patient Work-up" for new patients who will likely require platelet transfusions.
- This tests provides an HLA antigen type in case HLA matched platelet units are needed, as well as an HLA and platelet antibody screen/specificities.

What will we get when we order compatible units?
(assuming HLA antibodies only)

The HLA lab techs will make every attempt to provide the patient with:

- an HLA "A" match:
  all four antigens match
- "B1U" match:
  one antigen is unknown or blank
- or "B2U" match:
  two antigens are unknown or blank

- If an A, B1U or B2U is not available, then we will look for antigen negative units.
- If a unit is needed right away we can either do a STAT antibody screen or platelet crossmatch – depending on the patient need.

Why can't I always get an HLA matched unit quickly?

- The likelihood of finding an A match is very low, even with a large typed donor inventory.
- The likelihood of finding a B1U or B2U match is better, but also can be low depending on the HLA type of the patient.

Are antigen negative as good?

Studies have shown that use of the antibody specificity prediction method (ASP) or antigen negative units are just as effective as HLA matched platelets and better than cross-matched units.

It is all about type…. Hey baby!

Blood Bank, "My patient has an 80%PRA and HLA type = A01, A02; B08. We need regular transfusions….."

HLA Lab, "No Problem."

Blood Bank, "We need CMV negative units….."

HLA Lab, "Little Problem."

Blood Bank, "My patient has an 80% PRA and HLA type of A01, A25; B27, B44….."

HLA Lab, "I'm getting coffee."

Blood Bank, "Patient has platelet antibodies, …..GPIIa/IIIb"

HLA Lab, "Let me do a little investigation….. Could try crossmatching but HLA antigen neg might do just fine if they also have HLA antibodies."

There are times when we will set-up a crossmatch just to see if we can even pick-up the plt antibody….. Majority of the time we can't. The testing lab reports out levels out test kits can't detect.

Matched Platelet Strategies

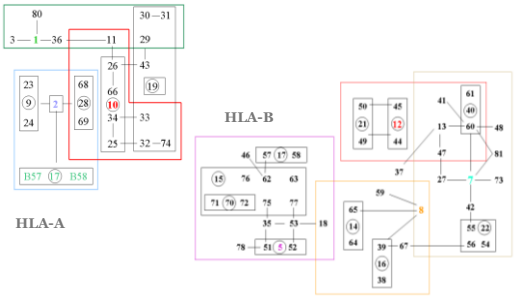
Unit selection based upon:

30-75% successes (~50%)
- Knowledge of HLA type
  - Search of unrelated donors for identical HLA-A & B type
  - >35% have no perfect match in most donor bases; >80% no match in inventory; other matches are "best guess"

50-75% successes (~55%)
- Crossmatching
  - React patient serum with platelets from inventoried units
  - Few antibodies, few units screened; many antibodies, many screened ($$$, test TAT & unit availability issues)

60-75% successes (~75%)
- Knowledge of HLA antibodies (± HLA type)
  - Provide units lacking specified HLA determinants
  - 1-2 orders of magnitude more donor / product matches

HLA Serological CREGs



HLA-Based Selection

Duquesnoy HLA-A & -B locus match determines outcome

[RECIPIENT: A1 A2 B7 B8]

- A — HLA identical all 4 loci [A1 A2 B7 B8]
- BU — All loci identical, only 3 Ag's detected [A1 B7 B8] B2U
  All loci identical, only 2 Ag's detected [A1 B8]
- BX — 3 loci identical, 4th cross-reactive [A1 A11 B7 B8] BUX
  3 Ag's, 2 identical, 3rd X-reactive [A1 A11 B8]
  B2X — 2 loci identical, 2 X-reactive [A1 A11 B7 B60]
- C — one locus mismatched [A1 A32 B7 B8]
- D — two or more loci mismatched, at least one NOT X-reactive [A1 A32 B7 B60]

Duquesnoy, RJ. *Am J Hematol* 1977;2:219-26.

Arrgg, no matches…what now?

HLA Lab can look at alternatives…

- Double check the antibodies specificity results… are we looking at transfusion data? If yes, then we could try units with conflicting antigens <5000/3000MFI.
- HLA B44/45, 8 have been shown to provide a decent increment even if the patient has an antiB44/45 or 8, why because the protein molecule is large and variable.
- C1q…. Assay only detects compliment binding.

Antigen-Negative Approach

Antibody Specificity Prediction (ASP)

(1621 tfxns. in 114 pts.)

– Post-tfxn.% plt recovery: ASP - 24.13% XM - 23.38%
HLA - 20.77% Rnd - 14.87%

– Markedly expands # of potential donors

	A matches	BU matches	ASP matches
Range	0 - 60	0 - 117	11 - 4638
Median	1	20	1365
		21	

– Perhaps as good as identical matches…

Pai SC. *Transfusion* 2010;50:2318-27; Petz LD. *Transfusion* 2000;40:1446-56.

Nothing is working…

- Pretreat with IVIG before transfusion. IVIG pretreatment can result in successful recovery after platelet transfusion in patients who are alloimmunized. Success rates vary (as much as 70%) and depend on the degree of alloimmunization. IVIG does not reduce the number of alloantibodies but does decrease platelet-associated immunoglobulins and possibly interferes with platelet destruction mechanisms. IVIG is more effective in improving short-term (1-6 h) recovery of platelets than platelet survival (>24 h).

Computer-Assisted HLA Selection

Duquesnoy abandoned his older CREG-matching schema for one more data-driven: ***HLAMatchmaker***

Each HLA antigen has shared immunogenic amino acid epitopes ("triplets" & "eplets"); patients cannot produce alloAb to their own HLA-A, -B & -C epitopes

- Donors without mismatched eplets are presumably fully compatible (A/BU-like)
- Inputting HLA type identifies alloantigens with the fewest mismatched eplets (i.e., BX/C matches more likely to succeed)
- Inputting results of the Ab ID, becomes a marginally-enhanced automated antigen-negative selector
  A/BU success rate ~85%, old CREG matching ~63% and enhanced HLAM ~84% 1-hr CCIs ≥ 7,500

Pai SC. *Transfusion* 2010;50:2318-27; Brooks, EG. *Transfusion* 2008;48:2159-66; Nambiar A. *Blood* 2006;107:1680-7

HLA Antibody Identification/Typing

Luminex-Based Antibody Identification/Typing

Lambda Array Beads
Multi-Analyte System (LABMAS)

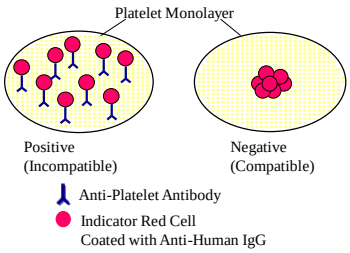
LABScan™ 200 Flow Analyzer, Luminex XY 96-sample reader and:

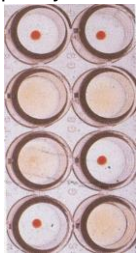
LABScreen® Single Antigen Beads
(~30 HLA-A / ~50 HLA-B Beads)

Uses PE-conj. goat anti-human IgG to detect antibody binding to beads (or PE-conj. C1q)

Platelet Crossmatching

Immucor Capture-P™ Solid Phase Red Cell Adherence (SPRCA) assay most frequently used.





Platelet Crossmatching

Detects HPA as well as HLA Ab, but also strong ABO Abs

- 2/3rds of O's have anti-A,B titers high enough to invalidate test

General correlation with the PRA, but misses some significant IgG & all IgM antibodies

- Up to 17% of significant AHG-CDC-detected antibodies may be missed in solid phase testing (i.e., product inappropriately appears compatible)

Crossmatch availability issues

- Limited or no night & weekend availability; 4-6 hour test TAT, limited products to test (ABO Identical)

Can get expensive

Petz LD. In: Murphy S, ed. *The HLA System: Basic Biology and Clinical Applications* Bethesda MD: AABB, 1999:133-175; Rachel JM. *Am J Clin Path* 1988;90:63-8

Management Pearls for Refractory Pts

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 3-5 days to get a recruited product to the hospital.

Management Pearls for Refractory Pts

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) antibodies.

- Consider brief support if IgM HLA antibodies suspected
- May succeed just because units are fresher & ABO-matched

Questions