Platelet Refractoriness and Fetal and Neonatal Alloimmune Thrombocytopenia: Approaches and Related Reference Services

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American Red Cross
Biomedical Services
Objectives

- To review platelet refractoriness and fetal and neonatal alloimmune thrombocytopenia
- To describe how serologic testing can aid clinical management and selection of blood products
- To describe how HPA genotyping can be helpful in differential diagnosis of FNAIT
- To describe how the platelet donors are screened to identify those whose platelet products may be useful for HPA alloimmunized patients
- To use cases to illustrate these points
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Platelet Refractoriness

- Thrombocytopenia – deficiency of platelets in the blood causing bleeding into the tissues, bruising and blood clotting after injury.

- Refractoriness is the repeated failure to achieve the desired level of platelets following transfusion
  - 20 – 70% of multi-transfused patients with thrombocytopenia experience a less-than-expected increase in platelet count
  - Patients treated for non-malignant hematopoietic disorders (anemia, hemophilia, etc.) are most likely to become refractory to platelet transfusions

- Cause of platelet refractoriness may be immune or non-immune
Immune Causes of Refractoriness

- HLA sensitization
  - presence of significant levels of antibodies to HLA Class I antigens in a refractory patient’s serum
  - the most common immune cause of platelet refractoriness
- Antibodies to HPA antigens
- ABO Incompatibility
- Antibodies in the sera of patients with congenital platelet glycoprotein deficiencies
  - ex - Glanzmann’s thrombasthenia – abnormality in genes for GPIIb/IIIa
Non-Immune Causes of Refractoriness

- Massive bleeding
- Fever
- Sepsis
- Splenomegaly
- Disseminated intravascular coagulation (DIC)
- Allogeneic transplantation
- Poor storage of platelets prior to transfusion
- Drugs
- Intravenous amphotericin B (suspected toxic effect on platelet function)
- Thrombotic thrombocytopenic purpura (TTP)
- Treatment regimen (such as chemotherapy)
- Liver dysfunction
Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT)

- A clinical condition caused by immune destruction of fetal platelets by maternal platelet antibody
- IgG antibody crosses the placenta, causing thrombocytopenia in the fetus or neonate
- Mother becomes sensitized to an incompatible fetal platelet antigen that the fetus inherited from the father
- 40-60% of FNAIT occurs in first pregnancy*
Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT): Symptoms

- Symptoms include petechiae, hematoma, severe bleeding, intracranial hemorrhage and death
- FNAIT is the leading cause of severe thrombocytopenia in the fetus and the leading cause of intracranial hemorrhage (ICH) full-term infants
  - Incidence is 0.3 to 1 in 1000
  - If untreated, 10-20% develop ICH (75% antenatally)
    - 15% fatality in FNAIT with ICH

https://casereports.bmj.com/content/casereports/2011/bcr.07.2011.4563/F4.large.jpg


https://casereports.bmj.com/content/casereports/2011/bcr.07.2011.4563/F4.large.jpg

Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT): Causes

Antibodies to HPA Antigens
- HPA-1a incompatibility between mother and fetus is the most commonly reported finding
  - Antibodies to other HPAs have also been implicated (e.g., HPA-2b, -3b, -5b, -15b)
- Only ~12% of HPA-1a negative mothers make anti-HPA-1a
- 90% of mothers with anti-HPA-1a carry HLA DRB3*01:01
  - While only 27% of general population carries this allele

Antibodies to ABO Antigens
- For example, Group O patients have large amounts of anti-A and anti-B IgG

Antibodies to HLA Antigens
- Platelets carry class I HLA antigens
Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT)

- Serologic diagnosis of FNAIT is determined by:
  - testing maternal serum for platelet antibodies using assays that can differentiate platelet-specific from non-platelet-specific reactivity
  - performing platelet genotyping on parental DNA
- Diagnosis is confirmed if HPA antibody is demonstrating in the maternal serum and there is incompatibility for the antigen on the paternal platelet
- Treatment for newborns includes administration of IVIG with or without antigen-compatible platelet transfusions
  - HPA negative platelets or washed maternal platelets
- Once a diagnosis of FNAIT has been made in a family, all future fetuses may be at risk
  - antenatal treatment with IVIG is an effective means of reducing fetal thrombocytopenia and reducing intracranial hemorrhage
FNAIT Diagnosis Algorithm, part I

FNAIT Diagnosis Algorithm, part II

The results of these tests can inform the risk and guide the clinical management of future pregnancy.

Other Causes of Thrombocytopenia in Neonates

- Infection
- Hypertension
- Autoimmune disorders
- Chronic hypoxia
- Necrotizing enterocolitis
- Thrombosis
- Congenital defect in platelet production
### for Recommendations FNAIT Interventions

<table>
<thead>
<tr>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>If a platelet transfusion is indicated, HPA-selected platelets should be used if immediately available.</td>
</tr>
<tr>
<td>If HPA-selected platelets are not immediately available, HPA unselected platelets should be transfused.</td>
</tr>
<tr>
<td>Platelets should be transfused immediately if life-threatening bleeding is present.</td>
</tr>
</tbody>
</table>
In the presence of life-threatening bleeding in a neonate such as intracranial or gastrointestinal bleeding, platelets should be transfused to maintain platelet counts initially above 100x10^9/L and then above 50x10^9/L for at least 7 days.

In the absence of life-threatening bleeding in a neonate such as intracranial or gastrointestinal bleeding, platelets should be transfused to maintain a platelet count above 30x10^9/L.

Antenatal IVIG administration to the mother commencing at 12-16 weeks gestation should be offered to all women in a subsequent pregnancy with maternal fetal incompatibility who have had a previous fetus or neonate with FNAIT related ICH.

For all other pregnancies with a previous neonate with FNAIT (without ICH), administering antenatal IVIG to the mother should be discussed prior to a subsequent pregnancy or when pregnancy with maternal fetal incompatibility is confirmed.

a. If antenatal intervention is required, IVIG administration to the mother should be started between 20 to 22 weeks (and not later than 24 weeks) gestation.

If the fetal platelet count is unknown, assisted delivery and invasive procedures on the fetus during delivery should be avoided including forceps, vacuum-assisted delivery, scalp blood sampling and scalp electrodes.
International Collaboration of Transfusion Medicine Guidelines (ICTMG) for FNAIT

- International team was comprised of adult and pediatric hematologists, maternal fetal medicine specialists, methodologists and transfusion medicine physicians
- Goal of developing a guideline for the management of FNAIT using a systematic approach and standardized method to optimize transfusion care
- Examined antenatal care, diagnostic tests including HLA genotyping and HPA alloantibody testing, post-natal interventions
3 Recent Systematic Reviews by ICTMG

**Antenatal management in fetal and neonatal alloimmune thrombocytopenia: a systematic review**

Dian Winkelhorst,1 Michael F. Murphy,2,3 Andreas Greinacher,4 Nadine Shehata,5,6 Taman Bakchoul,4,7 Edwin Massey,8 Jillian Baker,9 Lani Lieberman,10 Susano Tanael,11 Heather Hume,12 Donald M. Arnold,13 Shoma Baidya,14 Gerald Bertrand,15 James Bussel,16 Mette Kjaer,17,18 Cécile Kaplan,19 Jens Kjeldsen-Kragh,20 Dick Oepkes,1 and Greg Ryan21

**Postnatal intervention for the treatment of FNAIT: a systematic review**

Jillian M. Baker1 · Nadine Shehata2,3 · James Bussel4 · Michael F. Murphy5 · Andreas Greinacher6 · Taman Bakchoul6,7 · Edwin Massey8 · Lani Lieberman9 · Denise Landry10 · Susano Tanael11 · Donald M. Arnold11 · Shoma Baidya12 · Gerald Bertrand13 · Mette Kjaer14,15 · Cécile Kaplan16 · Jens Kjeldsen-Kragh15,17 · Dick Oepkes18 · Helen Savoia19 · Greg Ryan20 · Heather Hume21 · on behalf of the International Collaboration for Transfusion Medicine Guidelines (ICTMG)

**Maternal HPA-1a antibody level and its role in predicting the severity of Fetal/Neonatal Alloimmune Thrombocytopenia: a systematic review**

Mette Kjaer,1,2 Gerald Bertrand,3 Taman Bakchoul,4,5 Edwin Massey,6 Jillian M. Baker,7 Lani Lieberman,8 Susano Tanael,9 Andreas Greinacher,9 Michael F. Murphy,10 Donald M. Arnold,11 Shoma Baidya,12 James Bussel,13 Heather Hume,14 Cécile Kaplan,15 Dick Oepkes,16 Greg Ryan,17 Helen Savoia,18 Nadine Shehata,19,20 Jens Kjeldsen-Kragh,20 on behalf of International Collaboration for Transfusion Medicine Guidelines

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*J Perinatology* 2019. epub PMID: 30971767
Systematic review of 4 randomized controlled trials (RCT) and 22 non-RCT were used to assess antenatal treatment strategies for FNAIT

- Fetal blood sampling and intrauterine PLT transfusion had high (11%) complication rate including preterm delivery and fetal loss
- Noninvasive management of pregnant mother with history of FNAIT using weekly IVIg (typically 1g/kg,) was effective with 98.7% success for preventing intracranial hemorrhage
  - In cases with sibling with FNAIT (high risk), start IVIg between 12 and 20 weeks gestation
  - In cases without sibling with FNAIT (standard risk), start IVIg between 20 and 24 weeks
- No consistent evidence for value of adding steroids to maternal IVIg
- Paternal genotyping is recommended in suspected FNAIT (index case)
Systematic review of postnatal management of FNAIT

- Variables: HPA selected vs unselected platelets and/or IVIg
- Outcomes: platelet increments, hemorrhage and mortality

Findings

- HPA-selected platelets resulted in higher platelet increments and longer response times than unselected platelets
- Unselected platelets generally led to sufficient platelet increments to achieve clinical goals
- Platelet increments were not improved with IVIg
Systematic review of maternal Ab levels to HPA-1a to determine if the Ab level can be used to identify high-risk pregnancies

- Included prospective screening and retrospective studies of FNAIT cases
  - 8 of 10 retrospective studies used MAIPA and showed maternal Ab level correlated with severe thrombocytopenia. Not so for PSIFT or ELISA. Required similar timing with maternal and fetus/newborn sample collection
  - 3 prospective studies showed high (88-95%) negative predictive value but wide range (54-97%) of positive predictive value (PPV)
- Concluded that maternal Ab level could be used for risk stratification and planning route of delivery but more standardized prospective studies should be done to better assess PPV
Summary of ICTMG Findings

- Non-invasive management of pregnant mothers with prior history of NAIT using IVIG is effective; no evidence that steroids add benefit; paternal genotyping in index case
- HPA selected platelet transfusions were more effective than HPA-unselected platelets but unselected platelets were often effective to achieve clinical goals
- Maternal titers of anti-HPA-1a measured during the third trimester may be useful predictor of disease severity
Pregnant woman with previous FNAIT

Partner homozygous for implicated HPA

Partner heterozygous or unknown for implicated HPA

NIPT for HPA-1a; Amniocentesis for other HPAs

Fetus with implicated HPA antigen

No intervention required

Commence maternal antenatal intervention at 12 – 16 weeks age of gestation

Previously affected neonate with ICH

Consider maternal antenatal intervention at 20 – 22 weeks (and not later than 24 weeks) age of gestation

Partner homozygous for implicated HPA

Partner heterozygous or unknown for implicated HPA

NIPT for HPA-1a; Amniocentesis for other HPAs

Fetus with implicated HPA antigen

No intervention required

Yes

No

Yes

No

Fetal and Neonatal Alloimmune Thrombocytopenia: Recommendations for Evidence-Based Practice An International Approach
Lani Lieberman et al., for the International Collaboration for Transfusion Medicine Guidelines (ICTMG)
Thrombocytopenic neonate suspected of FNAIT

Manage as FNAIT without waiting for laboratory confirmation

Clinical suspicion of life threatening bleeding?

No

Cranial ultrasound within 24 hrs; Transfuse with available platelets (HPA selected or unselected) to maintain platelet count above 30 x 10^9/L; Follow up until platelets are normal in the absence of treatment.

Counseling for future pregnancies

Yes

Transfuse platelets immediately; (HPA selected or unselected) Maintain platelet count above 100 x 10^9/L initially and then above 50 x 10^9/L for at least 7 days; Cranial ultrasound within 24 hrs; Follow up until platelets are normal in the absence of treatment.

Counseling for future pregnancies

Fetal and Neonatal Alloimmune Thrombocytopenia: Recommendations for Evidence-Based Practice An International Approach. Lani Lieberman, A. et al., for the International Collaboration for Transfusion Medicine Guidelines (ICTMG)
Red Cross Standard Protocol for FNAIT

Red Cross Standard Protocol for FNAIT

Isolate Maternal PLT for Autocontrol and Paternal PLT for XM

ABO Type Maternal and Paternal RBCs, Maternal serum tests: PLT A/S, XM with Paternal PLT, Maternal PLT Autocontrol

Test Maternal Serum by Pak Lx

Local Lab performs only Solid Phase?

No

Yes

Retain aliquot of maternal sample, send remainder of clot tube and EDTA and paternal EDTA

Emergency Platelet Tx Need?

NO

YES

MD Consult

Antibody Identified?

No

Yes

Report Results, PLT Type incompatibilities and Tx Recommendation

*MD consult at any point for emergent need or transfusion recommendation

END

American Red Cross

Solid Phase – In most labs, this is Immucor Capture P A/S, Antibody Screen
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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 Ab detection, typing and platelet crossmatching
Luminex-Based Antibody Detection

Patient serum with HPA-specific Ab

PE-conjugated anti-human IgG

Wash beads

GP IIb/IIIa

HPA-1, -2, -3, -4, -5, GP IV and GPs IIb/IIIa, lb/IX, la/IIa
Platelet Suspension Immunofluorescence Test (PSIFT)

Indirect test using typed platelets (Ab detection)
Direct test using known antisera (typing)
Read visually, on a fluorometer or by flow
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Human Platelet Antigens

# Antigens, Glycoproteins and Genes

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Glyco-</th>
<th>HGNC</th>
<th>Chromosome</th>
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<td>HPA-15</td>
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<td>CD109</td>
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<td>2108C&gt;A</td>
<td>S682Y</td>
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</tbody>
</table>

https://www.ebi.ac.uk/ipd/hpa/freqs_1.html
HPA Genotyping

- Is used to assess incompatibility between partners
  - if woman has anti-HPA antibody or history of FNAIT
  - If platelet count is low and FNAIT is suspected in current fetus/newborn
- Typically performed on maternal and paternal samples and evaluated for incompatibility
- Can be performed on DNA from the fetus or newborn
  - standard sampling by fetal blood sampling (but with risk)
  - non-invasive prenatal testing (NIPT) via circulating cell-free fetal DNA from maternal plasma (ccffDNA)*

*not widely available
HPA Genotyping Assays

All “Research Use Only” (RUO)

- HPA-1a/1b SSP-PCR (LDT)
- HPA BeadChip (Immucor)
  - HPA-1,2,3,4,5,6,7,8,9,11,15
- Fluogene HPA (Inno-train)
  - HPA-1,2,3,4,5,6,9,15
- IDCore HPA (Grifols)
  - HPA-1,2,3,4,5,6,7,8,9,10,11,15
- HemoID HPA/HNA (Agena)
  - HPA-1,2,3,4,6,15
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- To use cases to illustrate these points
Supplying HPA Negative Platelets

- There is a need to have platelets available that lack specific HPA.
- Most commonly requested HPA-negative platelet product is HPA-1a negative.
- Serologic antigen typing or HPA-1a/b genotyping can be used to screen donors.
- Platelets found to be lacking HPA-1a are genotyped to determine their status of other clinically significant HPA antigens.
## HPA Genotype Frequencies

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype frequency* (%)</th>
<th>Whites</th>
<th>African Americans</th>
<th>Koreans</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-1a/1a (P^A1/P^A1)</td>
<td>80</td>
<td>84</td>
<td>99</td>
<td></td>
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<tr>
<td>HPA-1a/1b (P^A1/P^A2)</td>
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<td>16</td>
<td>1</td>
<td></td>
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<td><strong>HPA-1b/1b (P^M2/P^M2)</strong></td>
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<td>0</td>
<td>0</td>
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<tr>
<td>HPA-2a/2a (Ko²/Ko²)</td>
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<td>67</td>
<td>75</td>
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<td>HPA-2a/2b (Ko²/Ko¹)</td>
<td>12</td>
<td>30</td>
<td>24</td>
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<tr>
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<td>3</td>
<td>1</td>
<td></td>
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<tr>
<td><strong>HPA-3a/3a (Bak⁰/Bak⁰)</strong></td>
<td><strong>46</strong></td>
<td>40</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>HPA-3a/3b (Bak⁰/Bak⁰)</td>
<td>42</td>
<td>45</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>HPA-3b/3b (Bak⁰/Bak⁰)</td>
<td>12</td>
<td>15</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>HPA-4a/4a (Pen⁰/Pen⁰)</td>
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<td>100</td>
<td>100</td>
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<tr>
<td>HPA-4a/4b (Pen⁰/Pen⁰)</td>
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<td>0</td>
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<tr>
<td>HPA-4b/4b (Pen⁰/Pen⁰)</td>
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<td>0</td>
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<tr>
<td><strong>HPA-5a/5a (Br⁰/Br⁰)</strong></td>
<td><strong>79</strong></td>
<td>62</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>HPA-5a/5b (Br⁰/Br⁰)</td>
<td>19</td>
<td>34</td>
<td>6</td>
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<td>4</td>
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</tr>
</tbody>
</table>

* Genotyping was performed on DNA from 100 unrelated individuals in each group.

**HPA-1(a-)**
**HPA-2(b-)**
**HPA-3(b-)**
**HPA-5(b-)**

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Case Study #1

Clinical Presentation:
- Suspected NAIT
- 21 y/o Hispanic female, platelet count of 250 K/uL, not transfused in the previous three months
- Infant platelet count at birth was 25 K/uL - transfused with platelets – 15mL/kg
Testing to be Completed

- SPRCA – for antibody detection, compatibility testing and platelet antigen typing (HPA-1a), if requested
- Bead Immunoassay – to detect platelet and HLA Class I antibodies
- PSIFT – to verify platelet antigen typing and to detect serum and cell-bound antibodies directed against platelet surface markers
- ABO/Rh typing of maternal and paternal sample; antibody screen on maternal sample
- HPA genotyping panel
## Results

<table>
<thead>
<tr>
<th>SPRCA</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Screen HLA typed platelets + maternal serum</td>
<td>10/10 positive</td>
</tr>
<tr>
<td>Crossmatch Maternal serum + paternal platelets</td>
<td>Positive</td>
</tr>
<tr>
<td>Serological HPA-1a typing Paternal sample</td>
<td>HPA-1a Positive</td>
</tr>
<tr>
<td>Serological HPA-1a typing Maternal sample</td>
<td>Invalid – positive autogenic testing</td>
</tr>
</tbody>
</table>
## Results

<table>
<thead>
<tr>
<th>PSIFT</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody Panel</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum + HPA typed platelets</td>
<td>1/3 negative (negative plts were HPA-1a negative, positive platelets were HPA-1a positive)</td>
</tr>
<tr>
<td>Maternal serum + chloroquine treated platelets</td>
<td>Reactivity unchanged from untreated platelets</td>
</tr>
<tr>
<td><strong>Crossmatch</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum + paternal platelets</td>
<td>Positive</td>
</tr>
<tr>
<td>Maternal serum + chloroquine treated platelets</td>
<td>Positive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bead Immunoassay</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum</td>
<td>HPA-1a antibody detected</td>
</tr>
</tbody>
</table>
Results

- ABO testing
  - Probable ABO incompatibility – maternal sample is group O, antibody screen negative. Paternal sample is group A.

HPA Genotyping Panel

<table>
<thead>
<tr>
<th>HPA-1</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
<td>Mom</td>
<td>0</td>
<td>+</td>
<td>+</td>
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<td>0</td>
<td>+</td>
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</tr>
<tr>
<td>Dad</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
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<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>
Conclusion, Case 1

- Multiple HPA incompatibilities including HPA-1a
  - HPA-1a negative mother and HPA-1a positive father
    - 100% chance of his children being HPA-1a positive
- Maternal HPA-1a antibody detected
- HPA-1a negative platelet products available if needed
- All future pregnancies have potential for NAIT with this father
Case Study #2

Clinical Presentation:
- Suspected NAIT
- 38 y/o Hispanic female, not transfused in the previous three months
- Six prior pregnancies with previously affected infants
- Infant platelet count at birth was 107K/uL
# Results

<table>
<thead>
<tr>
<th>SPRCA</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Screen</td>
<td>10/10 positive</td>
</tr>
<tr>
<td>HLA typed platelets + maternal serum</td>
<td></td>
</tr>
<tr>
<td>Crossmatch</td>
<td>Positive</td>
</tr>
<tr>
<td>Maternal serum + paternal platelets</td>
<td></td>
</tr>
</tbody>
</table>
### Results

<table>
<thead>
<tr>
<th>PSIFT</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody Panel</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum + HPA typed platelets</td>
<td>3/3 positive</td>
</tr>
<tr>
<td>Maternal serum + chloroquine treated platelets</td>
<td>3/3 Positive</td>
</tr>
<tr>
<td><strong>Crossmatch</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum + paternal platelets</td>
<td>Positive</td>
</tr>
<tr>
<td>Maternal serum + chloroquine treated paternal platelets</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Bead Immunoassay</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum</td>
<td>Antibody to HPA-5b and HLA Class I antigens detected</td>
</tr>
</tbody>
</table>
Results

- ABO testing
  - maternal sample is Group A Positive
  - paternal sample is Group B Positive

HPA-1a/1b Genotyping

<table>
<thead>
<tr>
<th>ID</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mom</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Dad</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>
Conclusion, Case 2

- Antibody to HLA Class I antigens detected
  - recommend further testing to determine HLA specificity
- HPA-1b incompatibility noted, no antibody detected
- Antibody to HPA-5b detected
  - Panel of HPA-5b negative HLA compatible platelets recommended for crossmatching
  - HPA-5b negative platelets also offered for emergency
- Suspected ABO incompatibility
  - maternal ABO/Rh is A Positive (with anti-B)
  - paternal ABO/Rh is B Positive
Case Study #3

Clinical Presentation:

- Suspected NAIT
- 33 y/o Caucasian female, not transfused in the previous three months
- Infant platelet count at birth was 64K/uL
## Results

<table>
<thead>
<tr>
<th>SPRCA</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Screen HLA typed platelets + maternal serum</td>
<td>8/10 positive</td>
</tr>
<tr>
<td>Crossmatch Maternal serum + paternal platelets</td>
<td>Positive</td>
</tr>
</tbody>
</table>
# Results

<table>
<thead>
<tr>
<th>PSIFT</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody Panel</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum + HPA typed platelets</td>
<td>3/3 positive</td>
</tr>
<tr>
<td>Maternal serum + chloroquine treated platelets</td>
<td>3/3 negative</td>
</tr>
<tr>
<td><strong>Crossmatch</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum + paternal platelets</td>
<td>Positive</td>
</tr>
<tr>
<td>Maternal serum + chloroquine-treated paternal platelets</td>
<td>Negative</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bead Immunoassay</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum</td>
<td>Antibody to HLA Class I antigens detected</td>
</tr>
</tbody>
</table>
Results

- ABO testing
  - maternal sample is group A
  - paternal sample is group O

HPA Genotyping Panel

<table>
<thead>
<tr>
<th>HPA-</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>9</th>
<th>15</th>
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<tbody>
<tr>
<td>ID</td>
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<td>b</td>
<td>a</td>
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<td>a</td>
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<tr>
<td>Mom</td>
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<td>+</td>
<td>0</td>
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<tr>
<td>Dad</td>
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<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>
Conclusion, Case 3

- Maternal and paternal sample both type HPA-1a positive; incompatibility for HPA-15b
- No ABO incompatibility
- Antibody to HLA Class I antigens detected
  - recommended further testing to determine specificity of HLA reactivity
  - HPA-15b negative platelets could be used for crossmatching
Case Study #4

Clinical Presentation:

- Suspected NAIT
- 25 y/o Caucasian female, transfused with one unit of RBC in the previous three months
- Infant platelet count at birth was 37K/uL
# Results

<table>
<thead>
<tr>
<th>SPRCA</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Screen HLA typed platelets + maternal serum</td>
<td>10/10 negative</td>
</tr>
<tr>
<td>Crossmatch Maternal serum + paternal platelets</td>
<td>Positive</td>
</tr>
</tbody>
</table>
## Results

<table>
<thead>
<tr>
<th>PSIFT</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody Panel</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum + HPA typed platelets</td>
<td>3/3 negative</td>
</tr>
<tr>
<td>Maternal serum + chloroquine treated platelets</td>
<td>3/3 negative</td>
</tr>
<tr>
<td><strong>Crossmatch</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum + paternal platelets</td>
<td>Positive</td>
</tr>
<tr>
<td>Maternal serum + chloroquine-treated paternal platelets</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Bead Immunoassay</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal serum</td>
<td>No HLA or platelet antibodies detected</td>
</tr>
</tbody>
</table>
## Results – Additional Testing

<table>
<thead>
<tr>
<th>SPRCA</th>
<th>Result</th>
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<tbody>
<tr>
<td><strong>ABO Typed Platelets</strong></td>
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</tr>
<tr>
<td>Maternal Serum + Group B platelets</td>
<td>5/5 positive</td>
</tr>
<tr>
<td>Maternal Serum + Group O platelets</td>
<td>5/5 negative</td>
</tr>
</tbody>
</table>
Results

- ABO testing
  - maternal ABO/Rh is A Positive
  - paternal ABO/Rh is B Positive

HPA Genotyping Panel

<table>
<thead>
<tr>
<th>HPA-</th>
<th>1</th>
<th>2</th>
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<th>4</th>
<th>5</th>
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</tr>
<tr>
<td>Mom</td>
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<td>+</td>
<td>0</td>
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</tr>
</tbody>
</table>
Conclusion, Case 4

- Maternal and paternal sample both type HPA-1a positive
  - Incompatibility for HPA-3b, -5b

- No platelet or HLA antibodies detected

- Reactivity observed is consistent with the presence of platelet-reactive ABO antibody (anti-B)
Review of 4 years of FNAIT Testing in NRLST

Serologic Findings in 126 NAIT Cases

- 27% anti-HPA
- 41% HPA-1a
- 8% non-HPA-1a, ABO or HLA
- 19% HLA antibodies
- 25% ABO
- 3% Non-specific reactivity
- 4% No antibodies detected
Summary

- Platelet serologic testing can aid in differentiating immune from non-immune platelet refractoriness.
- Platelet serologic testing and HPA genotyping can aid in ruling in/out FNAIT.
- FNAIT testing can detect antibodies to HLA, ABO or HPA antigens with the most commonly reported incompatibility being anti-HPA-1a.
- Platelet product selection can be personalized based on the laboratory findings.
- For FNAIT, these findings also inform physicians for testing and treatment of future pregnancies.
Thank you for your attention!

Dexter.Facey@redcross.org
Margaret.Keller@redcross.org