Introduction to Neonatal Alloimmune Thrombocytopenia (NAIT)

Deborah Sage

Histocompatibility and Immunogenetics

Caring Expert Quality
Overview

• NAIT

• Platelets
  – Glycoproteins on platelets
  – Human Platelet Antigens (HPA)

• Sensitisation/Severity

• Laboratory tests

• Treatment/Management
NAIT

- Affects 1 in 1,000-2,000 live births
- Can be a cause of miscarriage
- Severe <50x10⁹/L
- Most common cause is HPA-1a antibodies
- Can affect first pregnancies (30%)
- 500-600 referrals/year
- 10% detection rate
Inheritance of Human Platelet Antigens

HPA-1b1b

HPA-1a1a

HPA-1b1a
How NAIT occurs

Maternal HPA-1a1a ANTIBODIES produced

Maternal antibodies

Platelet destruction resulting in thrombocytopenia
Clinical Impact and Diagnosis

• Can cause intracranial haemorrhage
  – Death
  – Developmental disabilities
  – Life-long social care
• Blood spots in baby
• Referral to test for maternal antibodies
• Future pregnancies!
• Cannot predict severity from current tests
Antigens on platelets

- ABO
- HLA class I antigens
- Platelet glycoproteins
  - GPIIb/IIIa
  - GPIa/IIa
  - GPIb/IX/V
  - CD109

Activated Platelet
Human Platelet Antigens (HPA)

There are currently 34 designated HPA systems.

- The majority (21) are associated with the GPIIb/IIIa complex.
- HPA are primarily di-allelic systems, i.e. result in a single amino acid substitution except HPA-14bw, which results from an ‘in frame’ deletion of three nucleotides.
- The ‘a’ allele is always the high frequency form and ‘b’ the low frequency. Three HPA systems have been shown to be tri-allelic; HPA-1c, -5c, -7cw but these mutations are very rare.
- A ‘w’ (workshop) assignment is given to systems where antibodies to only one antigen have been reported – this is the majority of recently identified HPA.
The most clinically significant platelet-specific alloantigens

<table>
<thead>
<tr>
<th>Allele</th>
<th>freq. (Cauc)</th>
<th>GP</th>
<th>Copies/cell</th>
<th>GP function</th>
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<tbody>
<tr>
<td>HPA-1a</td>
<td>84.5%</td>
<td>IIIa (CD61)</td>
<td>40K</td>
<td>Fg, vWF, Fn,</td>
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<tr>
<td>HPA-1b</td>
<td>15.5%</td>
<td></td>
<td></td>
<td>Coll, Vn</td>
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<tr>
<td>HPA-2a</td>
<td>89.9%</td>
<td>Ibα (CD42b)</td>
<td>20K</td>
<td>vWF</td>
</tr>
<tr>
<td>HPA-2b</td>
<td>10.1%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-3a</td>
<td>60.3%</td>
<td>IIb (CD41)</td>
<td>40K</td>
<td>Fg, vWF, Fn,</td>
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<tr>
<td>HPA-3b</td>
<td>39.7%</td>
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<td>Coll, Vn</td>
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<tr>
<td>HPA-4a</td>
<td>100%</td>
<td>IIIa (CD61)</td>
<td>40K</td>
<td></td>
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<tr>
<td>HPA-4b</td>
<td>0.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-5a</td>
<td>91.1%</td>
<td>la (CD49b)</td>
<td>2-4K</td>
<td>Collagen</td>
</tr>
<tr>
<td>HPA-5b</td>
<td>8.9%</td>
<td></td>
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</tr>
<tr>
<td>HPA-15a</td>
<td>50.0%</td>
<td>CD109</td>
<td>0.5 -2K</td>
<td>Collagen</td>
</tr>
<tr>
<td>HPA-15b</td>
<td>50.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Current Theory of Sensitisation

• Fetal platelets crossing the placenta
• β3 integrin present in saliva/sperm
• αVβ3 on trophoblasts of placenta
• Fetal maternal haemorrhage
Severity

- HPA-1a antibodies cause ICH
- HPA-5b are said not to be as severe NAIT
- HPA-3a antibodies reported to cause miscarriages
- αβ3 have been reported to cause ICH
- Cannot predict NAIT severity by lab tests
- Only predictor is subsequent pregnancies are more severe
Laboratory investigations (phase 1)

Routine investigation

• Screen of maternal serum versus typed HPA donor platelets (PIFT & MAIPA v panel of HPA-1, -2, -3, -4, -5, -6, -9, -15 typed platelets)

• Genotype (PCR-SBT) of maternal, paternal & infant sample

• Samples:
  – Maternal = 6ml EDTA & 6ml clot
  – Paternal = 6ml EDTA
  – Neonate = 1ml EDTA
Indirect Immunofluorescence Tests

1. Add test serum to HPA typed cell suspension.
2. Mix thoroughly & incubate.
3. Wash x 4.
4. Mix RPE/FITC labelled anti-IgG or IgM thoroughly & incubate in the dark.
5. Read in flow cytometer.
Advantages and disadvantages of indirect immunofluorescence tests

• **Advantages**
  - Sensitive, quick and cheap
  - Whole cell assays with potential to detect all antibodies to the membrane surface – important for some HPA, e.g. HPA-3a

• **Disadvantages**
  - May detect antibodies to HLA class I, ABH; IgG and immune complexes(?)
MAIPA assay

Solubilisation of platelets with detergent

Enzyme conjugated Goat anti-Human IgG

Incubated with enzyme substrate

Solid phase ELISA plate

Goat anti mouse capture antibody

Anti-GpIb/IX

Anti-HLA-1a

GpIb/IX

GpIIb/IIIa

Patient anti-HPA-1a

Patient anti-HLA

GpIIb/IIIa

Murine monoclonal anti-GpIIb/IIIa

Platelet of known HPA type incubated with patient serum

Colour change resulting in higher optical density (OD)
Each plate is laid out with glycoproteins from donor platelets in rows whilst test serum for each patient sample is in columns. The results are presented as an optical density, and a ratio of that OD to that of the corresponding negative control serum. An OD >0.150 and a ratio >3 normally constitute a positive.

We only use an anti-IgG conjugated antibody.
The advantages and disadvantages of the MAIPA assay

• **Advantages**
  – Specific and sensitive
  – Able to identify individual antibody specificities in complex antibody mixtures, differentiation from HLA class I antibodies

• **Disadvantages**
  – Need to know glycoprotein target antigen
  – Choice of monoclonal antibody can be critical
  – Solubilisation may modify the conformation of the native antigen
Laboratory investigations (phase 2)

Strong clinical evidence of NAIT or HPA-1b1b mother (HPA-1a antibodies not detected)

- Increase serum to cell ratio in PIFT & MAIPA
- Use different capture monoclonal antibodies
- HPA-1b1b, antibody negative women are monitored for antibody production during pregnancy.
- \textbf{DRB3}^*01:01 typing can be useful in cases to provide re-assurance if family is anxious.
- Use PakLx
- Crossmatch of maternal serum versus paternal platelets using PIFT and MAIPA assay.
- Demographics and reaction pattern
- GpIV antibody screening and typing
• Recombinant platelet glycoproteins captured on Luminex beads. Better specificity for defining HPA specific antibodies.

• Very sensitive and has picked up antibodies not detected in MAIPA.

• However potential for conformational changes as part of manufacturing process.

• More expensive than an in-house MAIPA.

• No CD109 (HPA-15).

• Labile glycoprotein that dissociates from platelets >24hrs. Requires fresh platelets.
Commercial bead based assay for the detection of HPA antibodies

- Detects antibodies against HPA-1, -2, -3, -4, -5, GPIV, HLA class I

**Advantages**

- Test results available after 3 hours
- 10uL of serum required
- Simple assay – beads + serum, wash, add conjugate, wash, test for bead associated fluorescence
- Sensitive for HPA-1a antibodies

**Disadvantages**

- Expensive
- Limited range of beads with antigen combinations
- Unable to detect antibodies to HPA-15
- Relatively insensitive to HPA-3a and HPA-5b antibodies compared to MAIPA
- Currently, cannot perform crossmatch or test for low frequency HPA

Porcelijn L et al. 54; 1486-92 (2014); Cooper N et al, Transfusion 56; 115-18 (2016)
Do we miss antibodies?

Yes

• The proportion of HPA-1b1b mothers in serologically negative NAIT cases is greater than expected

Why?

• Low affinity antibodies
• Isoforms of GPIIb/IIIa
• HPA-1a antibodies are polymorphic
Treatment

• During pregnancy
  – Intravenous immunoglobulin (IVIg)
  – Steroids
  – Platelet transfusions
  – Caesarean

• Following Birth
  – Platelet transfusions
Treatment of NAIT - Neonatal platelet transfusions
Patient ‘C.R.’ - Anti-HPA-1a

Days after birth

Platelet count (x 10^9/L)

Platelet Transfusions

IVIgG 0.4g/Kg/d

Random donor platelets
HPA-1a(-) platelets
Management of HPA alloimmunised women with heterozygous partners

• Current
  – Amniocentesis at ~15 weeks to determine HPA status of fetus
  – Chorionic villus sampling if earlier results required (e.g. if history of early fetal death)
  – HPA determined by PCR-SBT – preliminary result in 48-72 hours, cultured sample result at 14-21 days
  – Invasive procedure – spontaneous abortion & further alloimmunisation leading to increased disease severity

• Alternatives
  – Non-invasive, ffDNA typing for HPA-1 from maternal plasma available at some European centres - but earliest typing at 17 weeks and needs repeating later
Samples required for the investigation of NAIT

Samples required:

- Maternal 6mL EDTA anticoagulated blood
  6mL clotted
- Paternal 6mL EDTA anticoagulated blood (18mL for crossmatch)
- Baby 0.5 to 1mL EDTA anticoagulated blood

Maternal history:

- Ethnic origin
- Medication
- History of thrombocytopenia
- Infant platelet count
- Haemorrhage in infant
- Previous pregnancies - thrombocytopenia in infants?
- Previous transfusions
Thank you

Any questions?