The importance of platelet antigens and antibodies in immune-mediated thrombocytopenia

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2013.12.1
Agenda

1) Introduction to Platelet Antigens
   a) Platelet “non-specific” antigens
   b) Platelet “specific” antigens

2) Clinical Conditions involving Platelet Alloantibodies - Management
   a) Neonatal Alloimmune Thrombocytopenia (NAIT)
   b) Platelet Transfusion Refractoriness (PTR)
   c) Post-Transfusion Thrombocytopenic Purpura (PTP)

3) Activities of the ISBT Platelet Working Parties
   a) International WP
   b) Asia Regional WP
Human Platelet Antigens

1) Platelet “non-specific” alloantigens

- Major histocompatibility (MHC) molecules (HLA antigens)
  a) HLA class I (A, B, C) antigens expressed;
  b) Human platelets carry at least 20000 copies of class I HLA antigen;
  c) Homogeneous distribution per platelet;

- ABH blood group antigens
  a) Expressed at small quantities on platelets; however about 5% of normal subjects carry unusually large numbers of A and B antigen sites (“Type 2 high expressers”, up to 20000 antigen sites per platelet);
  b) ABH antigens also carried by GPIb, GPIIbIIIa, GPIaIIa, CD109, and glycolipids;
Human Platelet Antigens

1) Platelet “specific” alloantigens

a) GPIb-IX-V complex
   • GPIbα is found on platelets, megakaryocytes, vascular and tonsilar epithelium;

b) Integrin αIIbβ3 (GPIIbIIIa) complex
   • GPIIIa is found on platelets, megakaryocytes, monocytes, macrophages, endothelial and smooth muscle cells;

c) Integrin α2β1 (GPIaIIa) complex
   • GPIa is found on platelets, monocytes, B and T lymphocytes, NK cells, hematopoietic stem cells, vascular and thymic endothelial cells;
Human Platelet Antigens

d) CD109 (glycosylphosphatidylinositol (GPI)-anchored protein) of unknown function
  ● Only weakly expressed (about 1000 copies per platelet) and is relatively labile;

e) CD36 (GPIV)
  ● CD36 is found on platelets, megakaryocytes, red cells, monocytes, macrophages, erythroid precursors, adipocytes, activated keratinocytes, and some endothelial and epithelial cells;
  ● Member of the class B scavenger receptor family of proteins;
  ● About 5% of persons of African or Asian ancestry have inherited mutations leading to absence of CD36 expression (Type 2 CD36 deficiency), and are at risk of immunization;
Platelet membrane glycoproteins that carry HPA antigens

GPIaIIa  GPIIbIIIa  GPIbIX  CD109

# Nomenclature of Human platelet antigen (HPA)

<table>
<thead>
<tr>
<th>System</th>
<th>Ag</th>
<th>Original names</th>
<th>Frequency (%)</th>
<th>Glycoprotein</th>
<th>CD</th>
<th>Gene</th>
<th>Mutation</th>
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</table>

Alloimmunization to Platelet Antigens

1) Blood Transfusion
2) Pregnancy
3) Transplantation (Bone marrow, organ)

Produced alloantibodies bind to the target platelet alloantigen, leading to increased platelet sequestration via the reticuloendothelial system; intravascular platelet destruction due to complement activation is rare.
### Alloimmune Thrombocytopenias

#### Alloantigens implicated in alloimmune thrombocytopenia

<table>
<thead>
<tr>
<th>Antigen</th>
<th>NAIT</th>
<th>PTR</th>
<th>PTP</th>
<th>PAIT</th>
<th>TAATP</th>
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<tr>
<td>HPA</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>ABH</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
<td>(?)</td>
<td>(?)</td>
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<tr>
<td>Class I HLA</td>
<td>(+)?</td>
<td>(+)</td>
<td>(-)</td>
<td>(?)</td>
<td>(?)</td>
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1. Neonatal alloimmune thrombocytopenia (NAIT)
2. Platelet transfusion refractoriness (PTR)
3. Post-transfusion thrombocytopenic purpura (PTP)
4. Passive alloimmune thrombocytopenia (PAT)
5. Transplantation-associated alloimmune thrombocytopenia (TAATP)

Clinical conditions of Alloimmune Trombocytopenia

1) Neonatal Alloimmune Thrombocytopenia (NAIT)

Pathophysiology: Maternal sensitization to paternal antigen carried by fetal platelets lead to thrombocytopenia of the newborn;

Incidence: 1/1000 to 1/2000 births (Caucasian)
Neonatal Alloimmune Thrombocytopenia (NAIT)

Causative antibodies

1) **Anti-HPA antibodies**: mostly involved
2) **Anti-ABO antibodies**: rarely involved
3) **Anti-HLA I antibodies**: need to be confirmed

* HPA antigens are expressed as early as 16 weeks of gestation
Neonatal Alloimmune Thrombocytopenia (NAIT)

NAIT due to Anti-HPA Antibodies

Immune recognition of HPA alloepitopes during pregnancy is HLA restricted:

- Association of **HPA-1a** sensitization with **HLA-DR52** (DRB3*0101) alleles;
- Association of **HPA-5b** sensitization with **HLA-DR6** haplotype;
Neonatal Alloimmune Thrombocytopenia (NAIT)

NAIT due to ABO antibodies

- ABO antibodies of IgG type are necessary (usually blood group O individuals)
- The number of A, B, and H antigens per platelet is highly variable, but it is known that about 5% of normal subjects carry unusually large numbers of A and B antigen sites ("Type 2 high-expressers")
- ABO antibodies occur naturally, but rarely cause sequestration of ABO-incompatible platelets;
Neonatal Alloimmune Thrombocytopenia (NAIT)

NAIT due to anti-HLA Antibodies

- Cases of NAIT suspectedly due to anti-HLA are reported, but the association needs to be confirmed.
- Class I HLA Abs are found in about one third of multiparous women (15-31%), and anti-HPA Abs less frequently; however, platelet destruction is usually caused by the anti-HPA Abs.
- Protective immune mechanism of the placenta: anti-HLA antibodies adsorbed by the stromal cells of placenta expressing paternal antigens; routinely, the infants are born with normal platelet counts;
Symptoms

Neonates from mothers with antibodies:
- Asymptomatic (majority);
- Thrombocytopenia
- Severe thrombocytopenia (PLT<50x10⁹/L)
- Intracranial Hemorrhage (ICH):
  1) The most severe complication (incidence: 10-26%; 50-75% occurs intra-utero)
  2) Prognosis: about 10% die, and neurological sequelae develop in about 20%
  3) Recurrence rate of ICH in a subsequent pregnancy is about 80%

→ Preventive measures to avoid ICH are essential.

Neonatal Alloimmune Thrombocytopenia (NAIT)

Symptoms and Signs

- In contrast to maternal immunization to red cell antigens, it occurs during a first pregnancy;
- Frequently, the thrombocytopenia is mild (skin and mucous petechia), and the neonate remains asymptomatic;

Neonatal Alloimmune Thrombocytopenia (NAIT)

**Symptoms and Signs**

- Some cases develop severe thrombocytopenia (PLT < \(50 \times 10^9/\text{L}\)), and in 10-26%, intracranial hemorrhage (ICH) may develop, up to 80% of which occurring prenatally;

Transfontanelar ultrasound with hyperechogenic intracranial lesions (arrows) suggesting haemorrhage

Brain MRI  Axial T1WI (A) and T2WI (B): right parietal haemorrhage with blood in early and late subacute stage. Axial T2 gradient echo (C) and sagittal T1WI (D): there are signs of chronic bleeding, with haemosiderin deposits (C) and marked atrophy (D) of the cerebellar hemispheres and vermis.

Intracranial hemorrhage (ICH)

Axial and coronal sections of head computed tomography (CT) a case with massive ICH due to blood group anti-A antibody
Diagnosis of NAIT

In addition to the clinical symptoms of thrombocytopenia of the newborn, the following tests are important for the confirmation of diagnosis:

1) **Antibody detection** in maternal serum by the serological testing;
2) **Cross-match test** between maternal serum and paternal/newborn’s platelets;
3) **Genotyping of the parents and newborn** for the detection of incompatible single nucleotide polymorphism (SNP).
Laboratory Diagnosis of Platelet Alloantibodies

1) Binding Assays: PIFT/PSIFT, Flowcytometry

2) Antigen Capture Assays: MAIPA, MACE

3) Agglutination Assays: MPHA, M-MPHA, SPAA

4) Bead Assay: Luminex

5) Real time Assay: Surface Plasmon Resonance

PIFT/PSIFT: Platelet Adhesion/Suspension Immunofluorescence Test
MAIPA: Monoclonal Antibody Immobilization of Platelet Antigens
MPHA: Mixed Passive Hemagglutination/Magnetic-mixed Passive Hemagglutination
SPAA: Solid phase red cell adherence
**Diagnosis of NAIT**

### Antibody detection methods (gold standards)

<table>
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<tr>
<th>Region</th>
<th>Japan</th>
<th>US, Europe, Australia</th>
</tr>
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<tbody>
<tr>
<td>Methods</td>
<td>MPHA</td>
<td>PIFT</td>
</tr>
<tr>
<td></td>
<td>M-MPHA</td>
<td>MAIPA</td>
</tr>
</tbody>
</table>

No one of the presently available methods alone is able to detect all clinically relevant platelet antibodies.

- **Japan** ~50% “unresolved” cases
- **US, Europe** 20~70% the causative Ab not detected;

# Specificity of HPA Ab in NAIT (US)

<table>
<thead>
<tr>
<th>Single Ab specificity</th>
<th>Number of cases</th>
<th>(%)</th>
<th>Multiple Ab specificities</th>
<th>Number of cases</th>
<th>(%)</th>
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Davoren A, Curtis BR, Aster RH, McFarland JG. Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. Transfusion 2004; 44: 1220-25
### Specificity of HPA Ab in NAIT (Japan)

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Number of cases</th>
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The risk of ICH was the highest with anti-HPA-3.

Neonatal Alloimmune Thrombocytopenia (NAIT)

NAIT due to anti-HPA Antibodies (excluding “unresolved” cases):

- Caucasian
  1) Anti-HPA-1 (>80%)
  2) Anti-HPA-5 (10%)

- Japan
  1) Anti-HPA-4 (>50%)
  2) Anti-HPA-3a (15%)
  3) Anti-HPA-5 (10%)
## Antibody Detection in 24630 pregnant women in Japan

<table>
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<th>Antibody detection rate</th>
<th>Antibody specificity</th>
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<td>I</td>
<td>0.19% (19 / 9,750)</td>
<td>4b(4), 5a(2), 5b(13)</td>
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<td>II</td>
<td>1.14% (85 / 7,468)*</td>
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<td>&gt; III</td>
<td>1.75% (112 / 6,402)**</td>
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<td>Total</td>
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<td>4b(49), 5a(3), 5b(168), 4b+5b(1), Nak^a(2)</td>
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Ohto H, Miura S, Ariga H, Ishii T, Fujimori K, Morita S et al., Transf Med 2004; 14:399-408
## Diagnosis of NAIT
### Genotyping for the detection of incompatible SNP

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<th>Method</th>
<th>Pros</th>
<th>Cons</th>
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<td>Technically simple</td>
<td>Requires precise primer design</td>
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<td>Relatively inexpensive</td>
<td>Requires two reactions per assay sample</td>
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<td>Difficult to automate</td>
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<td>Subjective interpretation</td>
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<tr>
<td>PCR-RFLP</td>
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<td>SNP must create an allele-specific digestion site</td>
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<tr>
<td></td>
<td>Relatively inexpensive</td>
<td>Requires additional digestion step</td>
</tr>
<tr>
<td></td>
<td>Easier primer design</td>
<td>Cannot be automated</td>
</tr>
<tr>
<td></td>
<td>Less strict PCR reaction parameters</td>
<td>Subjective interpretation</td>
</tr>
<tr>
<td>TaqMan</td>
<td>Does not require additional handling after amplification</td>
<td>Probes expensive</td>
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<td>Real-time PCR assay</td>
<td>Automated allele discrimination</td>
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<td>Can be multiplexed</td>
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<tr>
<td></td>
<td>Easily interpreted</td>
<td></td>
</tr>
<tr>
<td>Bead array</td>
<td>Relatively automated</td>
<td>Expensive</td>
</tr>
<tr>
<td></td>
<td>Medium throughput</td>
<td>Requires test-specific technical expertise</td>
</tr>
<tr>
<td></td>
<td>Can be multiplexed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Relatively fast</td>
<td></td>
</tr>
<tr>
<td>All methods</td>
<td>Fresh platelet not required</td>
<td>Require test-specific technical expertise (newer methods);</td>
</tr>
<tr>
<td></td>
<td>Potential for automation (newer methods)</td>
<td>Mutations in the probe primer regions may result in false-negatives;</td>
</tr>
<tr>
<td></td>
<td>No reliance on antisera</td>
<td>Paternal low-frequency HPAs may not be detected</td>
</tr>
</tbody>
</table>
Laboratory Diagnostic of FNAIT: Algorithm

Suspected FNAIT → Maternal serum

- Antibody Positive: Antibody screening → incompatibility testing
  - Genotyping: Maternal vs. Paternal SNP
  - Genotyping: Maternal vs. Foetus SNP → FNAIT Confirmed

- Antibody Negative: Crossmatch: Maternal serum vs. Paternal platelets
  - Genotyping: Paternal and foetus SNP > rare or new SNP → FNAIT Confirmed
Prevention and Treatment of NAIT

→ Prevention of ICH is the most important

Antenatal

① Fetal blood sampling and intrauterine transfusion: due to the high risk of complications, their indication is mostly abandoned;
② Maternal administration of intravenous immunoglobulin (IVIG) ※ : almost 100% effective;
③ ② + Steroid : Platelet counts at birth improved;
④ Past history of newborn with ICH early start of treatment;
※ It is assumed that IVIG blocks FcR-mediated transplacental transport of pathological anti HPA antibodies and increases the clearance of anti-platelet antibodies (animal studies).

Prevention and Treatment of NAIT

→ Prevention of ICH is the most important

Post-natal

1) Transfusion of compatible platelets (maternal\* or HPA-compatible donor\**) for those with severe hemorrhage or thrombocytopenia (<50x10⁹/L);

* Remove plasma or replace with an additive solution and irradiate;

** ABO compatible, volume reduced, CMV negative and irradiated;

2) IVIG administration helpful if given prior to development of hemorrhage; help prolong survival of the incompatible PLTs;

3) Exchange transfusion: can be considered in symptomatic infants who do not respond to other treatments;

Cases with moderately severe thrombocytopenia (PLT 30-50x10⁹/L), without evident hemorrhage: can be managed with IVIG only.
Management of Subsequent Pregnancies

Confirmed fact: NAIT tends to be more severe in infants born subsequently to a mother who previously gave birth to an infant with the disease, especially with ICH;

1) Assess the risk of disease:
   - Paternal genotyping of the incompatible antigen:
     * **Homozygous:** 100% of chance of incompatibility;
     * **Heterologous:** 50% chance of incompatibility;
   - Fetal genotyping: amniotic fluid (18-20 weeks), chorionic villus material (8-10 weeks)

2) In case fetus is confirmed to be at risk for NAIT: estimate the likely of severity;
   - **Invasive method:** platelet count on a fetal blood sample (significant risk);
   - **Non-invasive method:**
     1. Consider the severity of disease in previously affected sibling;
     2. Test maternal serum for the strength of the anti-HPA antibody (not always predictive);
Non-invasive Management of FNAIT

- Treatment according to ICH in a previous sibling

**Previous ICH**

- Before 28 weeks of gestation: Initiate IVIG 2g/kg/week at 12 weeks of gestation with 1mg/kg/d prednisolone at 20-26 weeks until term
- At 28-36 weeks of gestation: Initiate IVIG 1g/kg/week at 12 weeks of gestation, increase IVIG to 2g/kg/week at 28-32 weeks with prednisolone 1mg/kg/day at 20-26 weeks until term

**Without ICH**

- PLT < 20x10⁹/L: IVIG 1g/kg/week with prednisolone 0.5mg/kg/day starting at 20 weeks until term
- PLT > 20x10⁹/L: IVIG 1g/kg/week or prednisolone 0.5mg/kg/day starting at 20 weeks until term

Non-invasive Management of FNAIT stratified according to the presence or absence of ICH, timing of its occurrence and degree of thrombocytopenia in a previous child

Salomon O and Rosenberg N. Br J of Haematol, 2013, 162, 304–312
“Unresolved Cases”
Causative antibody not determined

Possible Causes:

1) Technical problems:
   ● Low-avidity HPA antibodies;
   ● ”Labile antigens” (e.g., HPA-3)
   ● Methodologies
   ● Experience and expertise

2) Non-immunological causes?
   ● Splenomegaly, DIC, sepsis, drugs, etc.
   ● Hereditary thrombocytopenia
   ● Platelet dysfunction
Platelet Transfusion Refractoriness (PTR)

Definition

Lack of adequate post-transfusion corrected count increments (CCI) or percent platelet recovery (PPR) following at least 2 transfusions of fresh, random donor ABO-compatible platelets.

⇒ Important complication of blood transfusion, especially in those receiving multiple platelet transfusions.

Rebulla P. Haematologica 2005; 90: 247-253
Platelet Transfusion Refractoriness (PTR)

Etiology or PTR

Platelet Refractoriness

Immune factors (<20%)
- Alloimmune to HPA (10-20%)
  - Alloimmune to both (5%)

Non-immune factors (>80%)
- Alloimmune to HLA I (80-90%)
- Autoimmune (unknown)
- Sepsis, fever, DIC, splenomegaly, active bleeding, drugs, et.

Pavenski K et al. Tissue Antigens 2012; 79: 237-245
Platelet Transfusion Refractoriness (PTR)

Adverse outcomes

1) Longer hospital stays;
2) Higher inpatient hospital costs;
3) Inferior survival;
4) More bleeding, including fatal ones.

Pavenski K et al. Tissue Antigens 2012; 79: 237-245
Platelet Transfusion Refractoriness (PTR) Pathophysiology

1) Usually caused by class I HLA antibodies (HLA-A, -B, -C), and more rarely by HPA antibodies; majority are IgG;

2) Repeated antigenic exposure through transfusions of blood containing leukocytes leads to production of HLA antibodies in approximately 50-90% of all cases;

3) HLA and HPA antibodies are bound to the macrophage Fc receptor and platelets are destroyed in the spleen, shortening the lifetime of transfused platelets;
**Platelet Transfusion Refractoriness (PTR)**

**TRAP Study**

Multicenter randomized controlled trial to reduce alloimmunization against platelets (1997)

<table>
<thead>
<tr>
<th>603 Patients</th>
<th>Controls: untreated pooled random donor platelets</th>
<th>Leukoreduced pooled random donor platelets</th>
<th>Leukoreduced single-donor apheresis platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>131</td>
<td>137</td>
<td>132</td>
</tr>
<tr>
<td>Alloimmunization</td>
<td>45%</td>
<td>18% *</td>
<td>17% *</td>
</tr>
<tr>
<td>Refractoriness</td>
<td>16%</td>
<td>7% *</td>
<td>8%</td>
</tr>
<tr>
<td>Alloimmunization and refractoriness</td>
<td>13%</td>
<td>3% *</td>
<td>4% *</td>
</tr>
</tbody>
</table>

* Statistical significance compared to control group

Either leukoreduced or UVB irradiation effectively prevented alloantibody-induced refractoriness;

### Platelet Transfusion Refractoriness (PTR)
Universal prestorage leukoreduction (late 1990s ~)

<table>
<thead>
<tr>
<th></th>
<th>Pre-universal PSLR group (%)</th>
<th>Post-universal PSLR group (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall refractoriness</td>
<td>27/315 (40)</td>
<td>68/302 (23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Overal alloimmune refractoriness</td>
<td>44/315 (14)</td>
<td>12/302 (4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Universal PSLR significantly reduced both the incidence of alloimmunization and alloimmune refractoriness in chronically transfused hematological patients.

Platelet Transfusion Refractoriness (PTR) Prevention

1) Use of ABO-matched, single-donor platelets;
2) Prophylactic HLA-matching;
3) Leukoreduction or UV irradiation of cellular blood products, including red cells;

Pavenski K et al. Tissue Antigens 2012; 79: 237-245
Platelet Transfusion Refractoriness (PTR)

Management

1) Modulation of the patient’s immunological response to foreign HLA:
   - Administration of intravenous immunoglobulin (IVIG);
   - Plasmapheresis;

2) Selection of transfusion donor/product factors:
   - Selection of donors with HLA “matched”.

Pavenski K et al. Tissue Antigens 2012; 79: 237-245
Platelet Transfusion Refractoriness (PTR) Management

1) Antibody specificity prediction or antigen-negative approach:
   - Provision of platelets that contain no antigens reactive with the alloantibody formed by the patient;
   - The precise determination of HLA antibody specificity is required;

2) Platelet cross-matching:
   - Provides a faster and more effective alternative to HLA matching;

Reviewed in Pavenski K et al. Tissue Antigens 2012; 79: 237-245
Management Algorithm

Hod E and Schwartz J. Br J Haematol 2008; 142: 348-360
Platelet Transfusion Refractoriness (PTR)
The Japanese Approach

Prevention

1) ABO compatible, single-donor apheresis-derived platelet products are routinely used; → reduced alloantigen exposure;

2) Universal pre-storage leukoreduction is implemented; → reduced alloimmunization risk due to donor leukocytes;

3) The validity date is 4 days after the collection, shorter than in other countries (mostly between 5-7 days).
Platelet Transfusion Refractoriness (PTR)  
The Japanese Approach

Management

3) Patients are routinely screened for HLA/HPA antibodies, and the specificity determined in case antibody is detected;

4) HLA- or HPA-matched single-donor platelets are provided (at request); → HLA-typed donor registry established at the Japanese Red Cross Blood Center;

5) Platelet cross-match performed to confirm compatibility;
Post-transfusion Thrombocytopenic Purpura (PTP)

Pathophysiology

1) Extremely rare complication of blood transfusion, characterized by a sudden episode of severe thrombocytopenia occurring approximately a week (5-14 days) after a platelet-containing transfusion;

   - Incidence: 1:50,000 – 100,000 transfusions (suggested)
   - 1:24,000 blood components transfused (1)

2) Propensity background: mostly female patients (about 90%) who had been previously immunized against HPA during pregnancy or transfusion, and are re-exposed to the HPA alloantigen by blood transfusion;

Rozman P. Transplant Immunology 2002; 10: 165-181
1) Shtalrid M et al. IMAJ 2006; 8: 672-674
Post-transfusion Thrombocytopenic Purpura (PTR) Causative antibody

1) Originally believed to be restricted to HPA-1a-negative women previously immunized by a HPA-1a-positive pregnancy; now it is confirmed to be caused also by HPA-1b, -2b, -3a, -3b, -4a, -5a and -5b;

2) During thrombocytopenic phase, pan-specific antibodies of IgG and IgM against GPIIbIIIa, GPIb-IX, and GPIaIIa were formed together with HPA alloantibodies; these panreactive antibodies were suggested as the responsible for the autologous platelet destruction (1);

Rozman P. Transplant Immunology 2002; 10: 165-181
1) Taaning E et al. Vox Sang 1999; 76(2): 120-123
Post-transfusion Thrombocytopenic Purpura (PTR)  

Clinical course

1) **Symptoms:** purpura, cutaneous bleeding, epistaxis, gastrointestinal hemorrhage, etc.

2) The clinical course may be severe;

3) **Mortality rate:** 10-20%;

4) **Correct diagnosis and immediate treatment are essential.**

5) **Diagnosis:** identification of the causative HPA antibody + platelet genotyping;

Shtalrid M et al. IMAJ 2006; 8: 672-674
Post-transfusion Thrombocytopenic Purpura (PTR) Management

1) **Treatment of choice**: high dose immunoglobulin administration (IVIG) with or without steroids

2) **Plasmapheresis** for the removal of the causative antibody;

3) **Transfusion of platelets** lacking the responsible antigen: in rare cases of life-threatening hemorrhage, it may temporarily increase platelet count, stop the bleeding and save life.

Shtalrid M et al. IMAJ 2006; 8: 672-674
Post-transfusion Thrombocytopenic Purpura (PTR)  Differential diagnosis

1) Heparin-induced thrombocytopenia (HIT)
   - Whereas PTP is characterized by very low platelet count ($<15 \times 10^9/L$), with severe hemorrhagic symptoms, HIT have higher platelet counts (usually $>20 \times 10^9/L$), with severe hemorrhagic symptoms;

2) Other non-immune causes of thrombocytopenia
   - The identification of the causative HPA alloantibody can help differentiate.

Shtalrid M et al. IMAJ 2006; 8: 672-674
Platelet Immunobiology Working Parties of the ISBT

Chair: Dr. Sentot Santoso (Giessen University)  
Co-chair: Dr. Nelson H. Tsuno (The University of Tokyo)
Objectives of Platelet Immunobiology Working Parties

* Develop and improve methodologies for platelet antigen/antibody testing;
* Share and exchange of knowledge among the participant labs;
* Stimulate collaborative studies;
* Exchange of materials (rare antiserum, rare platelets);
* Validation of standard reagents and methodologies;
* **Provide feed-back to the clinicians.**
2012: Among the 34 labs, only 4 (2 Countries) were from Asia
## Distribution of HPA alleles among South-Asian and Caucasian

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>1a</th>
<th>1b</th>
<th>2a</th>
<th>2b</th>
<th>3a</th>
<th>3b</th>
<th>4a</th>
<th>4b</th>
<th>5a</th>
<th>5b</th>
<th>6a</th>
<th>6b</th>
<th>15a</th>
<th>15b</th>
</tr>
</thead>
<tbody>
<tr>
<td>China (Feng et al., 2006)</td>
<td>1000</td>
<td>0.994</td>
<td>0.006</td>
<td>0.951</td>
<td>0.049</td>
<td>0.597</td>
<td>0.406</td>
<td>0.955</td>
<td>0.005</td>
<td>0.986</td>
<td>0.014</td>
<td>0.986</td>
<td>0.014</td>
<td>0.532</td>
<td>0.468</td>
</tr>
<tr>
<td>Indonesian (Asmarinah et al., 2013)</td>
<td>500</td>
<td>0.970</td>
<td>0.030</td>
<td>0.940</td>
<td>0.060</td>
<td>0.520</td>
<td>0.480</td>
<td>0.950</td>
<td>0.050</td>
<td>0.970</td>
<td>0.030</td>
<td>0.950</td>
<td>0.050</td>
<td>0.510</td>
<td>0.490</td>
</tr>
<tr>
<td>Indonesian (Liu et al., 2002)</td>
<td>107</td>
<td>0.991</td>
<td>0.009</td>
<td>0.939</td>
<td>0.061</td>
<td>0.505</td>
<td>0.495</td>
<td>1.000</td>
<td>0.000</td>
<td>0.995</td>
<td>0.005</td>
<td>0.967</td>
<td>0.033</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Japan (Tanaka et al., 1996)</td>
<td>73</td>
<td>0.998</td>
<td>0.020</td>
<td>0.900</td>
<td>0.100</td>
<td>0.718</td>
<td>0.282</td>
<td>0.989</td>
<td>0.011</td>
<td>0.973</td>
<td>0.027</td>
<td>0.973</td>
<td>0.027</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Korea (Seo et al, 1998)</td>
<td>200</td>
<td>0.988</td>
<td>0.012</td>
<td>0.923</td>
<td>0.077</td>
<td>0.555</td>
<td>0.445</td>
<td>0.990</td>
<td>0.010</td>
<td>0.978</td>
<td>0.022</td>
<td>0.980</td>
<td>0.020</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malaysia (Tan et al., 2012)</td>
<td>200</td>
<td>0.975</td>
<td>0.025</td>
<td>0.963</td>
<td>0.037</td>
<td>0.503</td>
<td>0.497</td>
<td>0.995</td>
<td>0.005</td>
<td>0.950</td>
<td>0.050</td>
<td>0.993</td>
<td>0.007</td>
<td>0.515</td>
<td>0.485</td>
</tr>
<tr>
<td>Taiwan (Shih et al., 2003)</td>
<td>300</td>
<td>0.997</td>
<td>0.003</td>
<td>0.960</td>
<td>0.040</td>
<td>0.757</td>
<td>0.425</td>
<td>0.998</td>
<td>0.002</td>
<td>0.985</td>
<td>0.015</td>
<td>0.963</td>
<td>0.037</td>
<td>0.538</td>
<td>0.462</td>
</tr>
<tr>
<td>Thai (Kupatawintu et al., 2005)</td>
<td>500</td>
<td>0.985</td>
<td>0.015</td>
<td>0.952</td>
<td>0.048</td>
<td>0.560</td>
<td>0.440</td>
<td>1.000</td>
<td>0.000</td>
<td>0.968</td>
<td>0.032</td>
<td>0.986</td>
<td>0.014</td>
<td>0.491</td>
<td>0.509</td>
</tr>
<tr>
<td>Vietnam (Halle et al., 2004)</td>
<td>120</td>
<td>0.986</td>
<td>0.014</td>
<td>0.953</td>
<td>0.047</td>
<td>0.486</td>
<td>0.514</td>
<td>1.000</td>
<td>0.000</td>
<td>0.972</td>
<td>0.028</td>
<td>0.986</td>
<td>0.014</td>
<td>0.477</td>
<td>0.523</td>
</tr>
<tr>
<td>Caucasian (Jones et al., 2003)</td>
<td>134</td>
<td>0.844</td>
<td>0.160</td>
<td>0.925</td>
<td>0.075</td>
<td>0.627</td>
<td>0.373</td>
<td>1.000</td>
<td>0.000</td>
<td>0.914</td>
<td>0.086</td>
<td>1.000</td>
<td>0.000</td>
<td>0.524</td>
<td>0.476</td>
</tr>
</tbody>
</table>

HPA-4 system seems to be important, in addition to Japan, in South Korea and Indonesia.
HPA-6 system seems to be important in Asia.

Antigens of importance in Asia
Different from Caucasian

CD36 (Nak-a antigen)-negative
1) Incidence: Asian (5-10%), African Americans (2.4%)
2) Types:
   ● Type I deficiency: CD36 absent from both platelets and monocytes (0.54% in Japan (2), 0.5% in China)
     → May produce anti-CD36 isoantibodies after transfusion or pregnancy;
   ● Type II deficiency: CD36 absent only from platelets (4.0% in Japan (2), 1.3% in China (5))
3) Clinical significance: CD36 isoantibodies involved in the pathophysiology of NAIT (1), PTR (2,3), and TRALI (4).

Ethnic differences exist in HPA frequency distribution between Caucasian and Asian populations;
- Different HPA types may be involved in clinical conditions (NAIT, PTR, PTP);
- HPA types specific for Asian populations eventually may exist;
- The preferred methodologies for platelet serology is different between US/Europe/Australia and some Asian Countries;
* June 2010: the proposal for the establishment of the Platelet Working Party in Asia was approved during the ISBT meeting in Berlin;
* November 2010: the first training course on platelet immunology methods was organized at the University of Tokyo (Japan); 7 people from 5 countries attended;
* November 2011: the first workshop was organized (ISBT Regional Congress, Taipei); 13 labs participated;
* May 2013: the second training course on platelet immunology methods organized at the Guangzhou blood center (China); 17 people from 8 countries attended;
* December 1<sup>st</sup> 2013: the 2<sup>nd</sup> Workshop of Asia Regional was organized during the ISBT Regional meeting in Kuala Lumpur; 20 labs from 11 countries attended;

Provided Exercises

1) Detection and identification of anti-platelet antibodies by the MAIPA, MPHA and/or PIFT and/or others;
2) Genotyping of the provided samples for HPA;
3) Determination of the frequency of CD36-negative by PIFT.
International Platelet Immunology Workshop

1982  Budapest (1st)
1984  Munich (2nd)
1986  Sydney (3rd)
1988  London (4th)
1990  Los Angeles (5th)
1992  Anaheim (6th)
1994  Amsterdam (7th)
1996  Makuhari (8th)
1998  Oslo (9th)
2000  Vienna (10th)
2002  Vancouver (11th)
2004  Edinburgh (12th)
2006  Cape Town (13th)
2008  Macao (14th)
2010  Berlin (15th)
2012  Cancun (16th)

Serological typing

DNA typing

Workshop in Cancun
Participating labs: 34

Participating labs: 20

Asia Regional

1st  Taipei (2011)
2nd  Kuala Lumpur (2013)