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

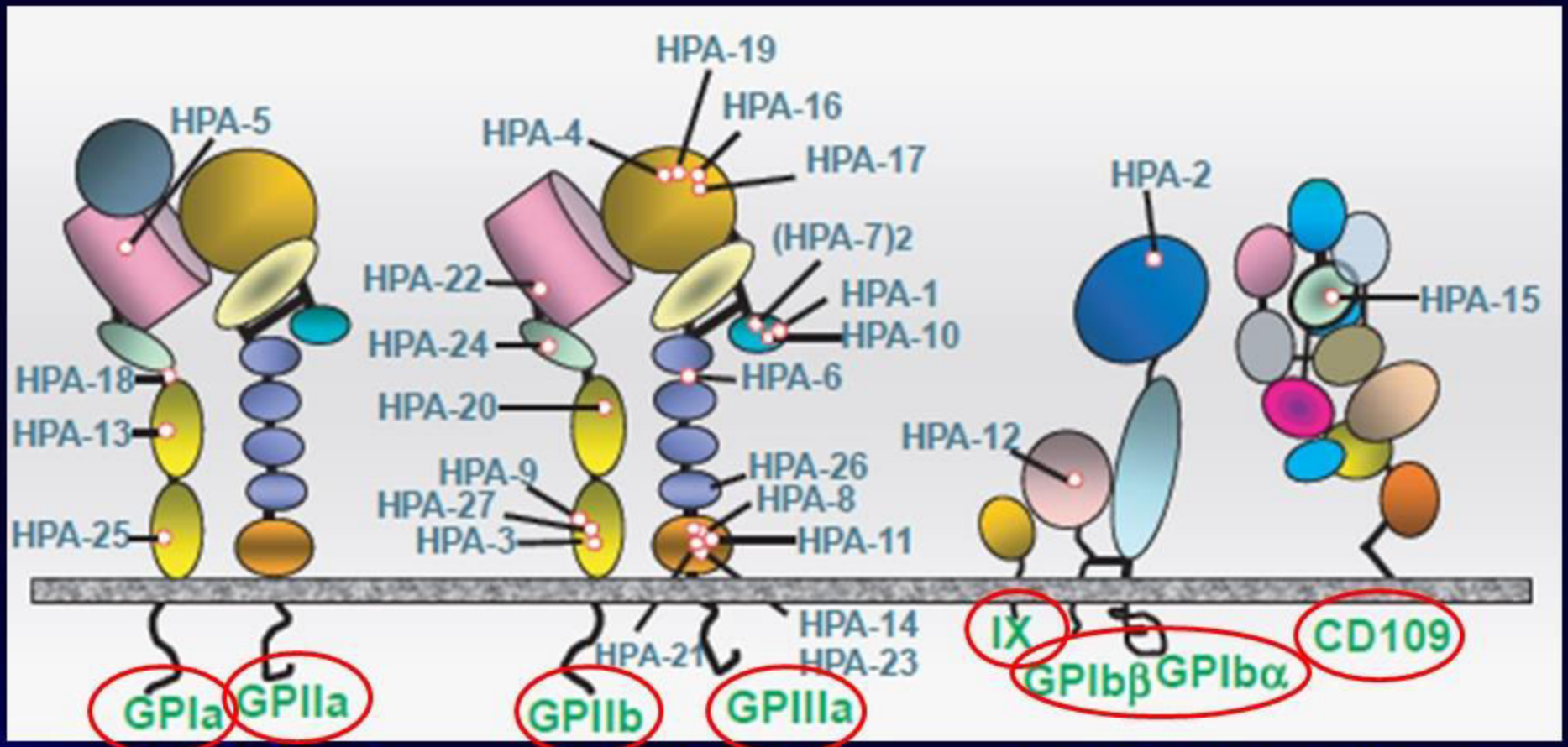
d) CD109 (glycosylphosphatidylinositol (GPI)-anchored protein) of unknown function

- 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**

Peterson JA, McFarland JG, Curtis BR, Aster RH. Neonatal alloimmune thrombocytopenia: pathogenesis, diagnosis and management. Br J Haematol 2013; 161: 3-14

Nomenclature of Human platelet antigen (HPA)

System	Ag	Original names	Frequency (%)		Glycoprotein	CD	Gene	Mutation	aa Exchange (Protein)
			Japane	Caucas					
HPA-1	HPA-1a	Zw^{a}, PI^{A1}	>99	98	GPIIIa	CD61	*ITGB3*	176T>C	L33P
	HPA-1b	Zw^{b}, PI^{A2}	<1	29					
HPA-2	HPA-2a	Ko^{b}	98	>99	GPIbα	CD42b	*GP1BA*	482C>T	T145M
	HPA-2b	Ko^{a}, Sib^{a}	25	13					
HPA-3	HPA-3a	Bak^{a}, Lek^{a}	79	81	GPIIb	CD41	*ITGA2B*	2621T>G	I843S
	HPA-3b	Bak^{b}	71	70					
HPA-4	HPA-4a	Yuk^{b}, Pen^{a}	>99	>99	GPIIIa	CD61	*ITGB3*	506G>A	R143Q
	HPA-4b	Yuk^{a}, Pen^{b}	2	<1					
HPA-5	HPA-5a	Br^{b}, Zav^{b}	>99	99	GPIa	CD49b	*ITGA2*	1600G>A	E505K
	HPA-5b	Br^{a}, Zav^{a}, Hc^{a}	8	20					
	HPA-6bw	Ca^{a}, Tu^{a}	3	<1	GPIIIa	CD61	*ITGB3*	1544G>A	R489Q
	HPA-7bw	Mo^{a}	<1	<1	GPIIIa	CD61	*ITGB3*	1297C>G	P407A
	HPA-8bw	Sr^{a}	<1	<1	GPIIIa	CD61	*ITGB3*	1984C>T	R636C
	HPA-9bw	Max^{a}		1	GPIIb	CD41	*ITGA2B*	2602G>A	V837M
	HPA-10bw	La^{a}		1	GPIIIa	CD61	*ITGB3*	263G>A	R62Q
	HPA-11bw	Gro^{a}		<1	GPIIIa	CD61	*ITGB3*	1976G>A	R633H
	HPA-12bw	Iy^{a}		<1	GPIbβ	CD42c	*GP1BB*	119G>A	G15E
	HPA-13bw	Sit^{a}		<1	GPIa	CD49b	*ITGA2*	2483C>T	T799M
	HPA-14bw	Oe^{a}		<1	GPIIIa	CD61	*ITGB3*	1909-1911delAAG	K611del
HPA-15	HPA-15a	Gov^{b}	75	81	CD109	CD109	*CD109*	2108C>A	S682Y
	HPA-15b	Gov^{a}	76	60					
	HPA-16bw	Duv^{a}		<1	GPIIIa	CD61	*ITGB3*	497C>T	T140I
	HPA-17bw	Va^{a}		<1	GPIIIa	CD61	*ITGB3*	662C>T	T195M

P. Metcalfe, N. A. Watkins, W. H. Ouwehand, C. Kaplan,* P. Newman, R. Kekomaki, M. de Haas, R. Aster, Y. Shibata, J. Smith, V. Kiefel & S. Santoso. Nomenclature of human platelet antigens Vox Sanguinis (2003) 85, 240–245

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					
Antigen	**NAIT**	**PTR**	**PTP**	**PAIT**	**TAATP**
HPA	(+)	(+)	(+)	(+)	(+)
ABH	(+)	(+)	(-)	(?)	(?)
Class I HLA	(+)?	(+)	(-)	(?)	(?)

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)

Salama A. Alloimmune thrombocytopenia. J Pediatr Hematol Oncol 2003; 25 (Suppl 1): S39-S41

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;

NAIT

Symptoms

Neonates from mothers with antibodies:

- Asymptomatic (majority) ;
- Thrombocytopenia
- Severe thrombocytopenia (PLT<50x10^9/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%;

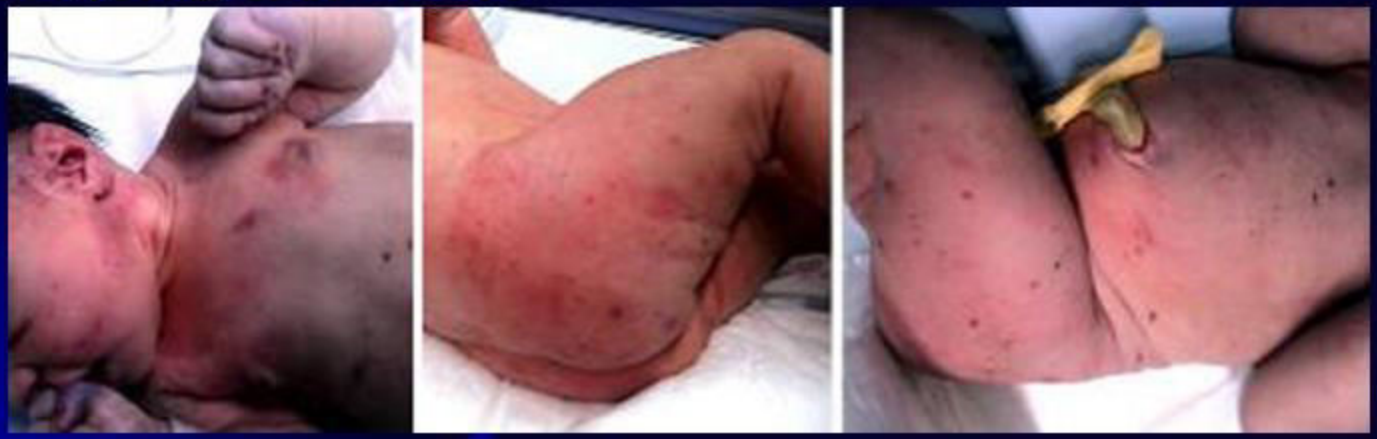
→ **Preventive measures to avoid ICH are essential.**

Aromsburg SA, Shaz BH, Westhoff C, Cushing MM. Determination of human platelet antigen typing by molecular methods: importance of diagnosis and early treatment of neonatal alloimmune thrombocytopenia. Am J Hematol. 87: 525-8, 2012

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**;

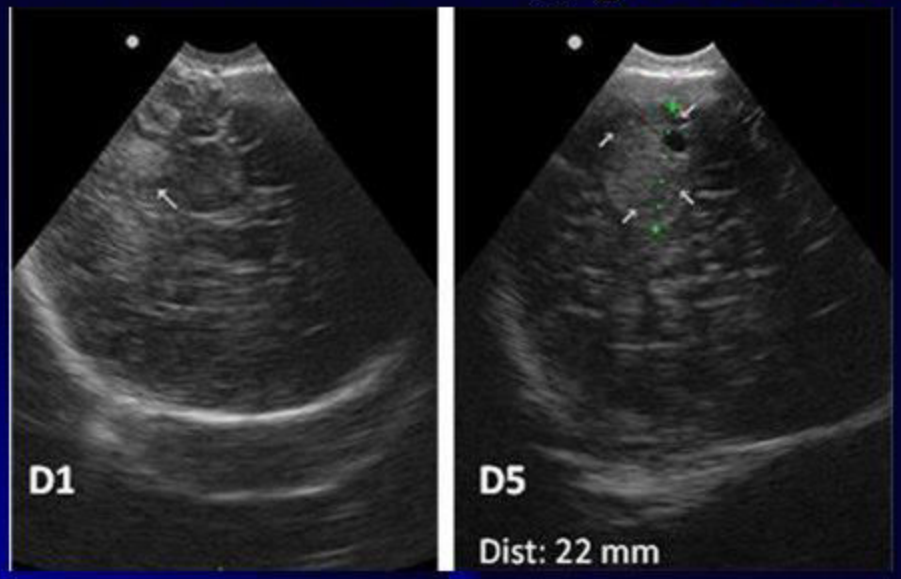


Silva F, et al. Severe intracranial haemorrhage in neonatal alloimmune thrombocytopenia. BMJ Case Reports 2011; doi:10.1136/bcr.07.2011.4563

Neonatal Alloimmune Thrombocytopenia (NAIT)

Symptoms and Signs

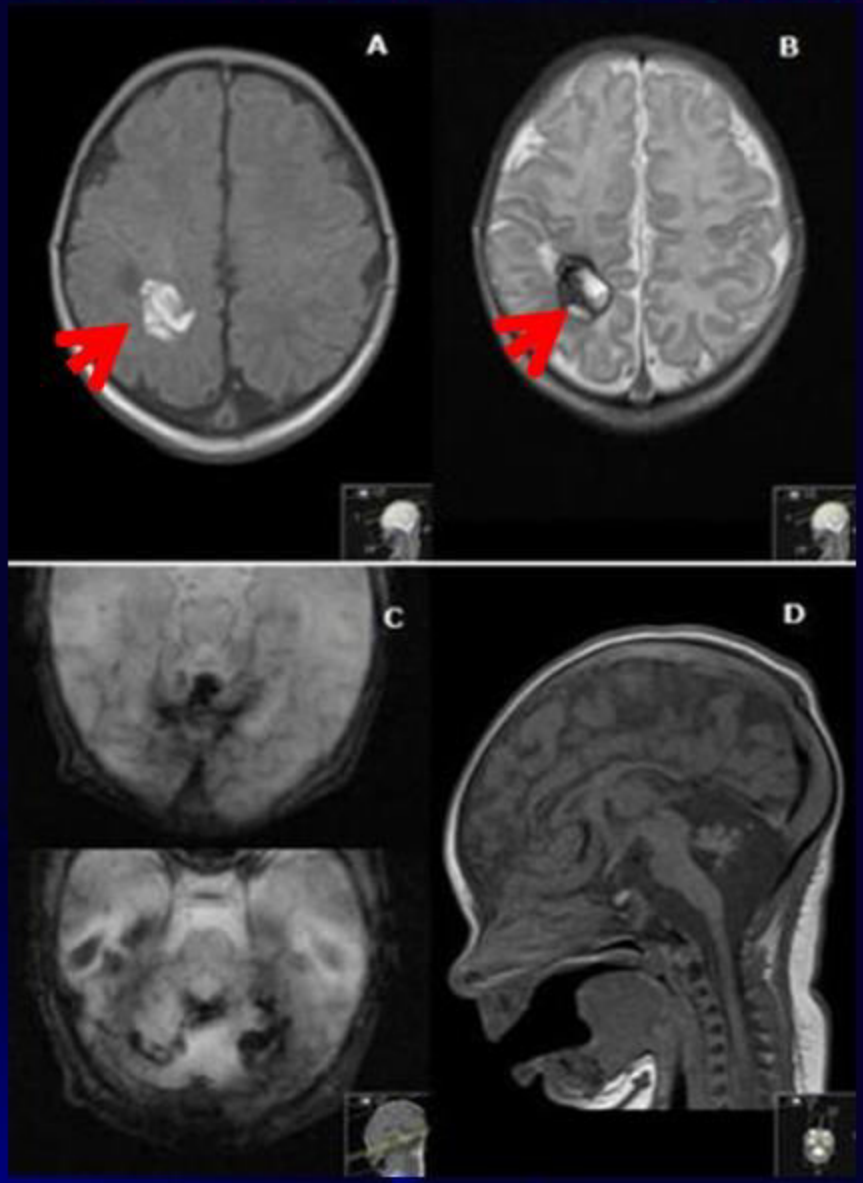
- Some cases develop severe thrombocytopenia (PLT < $50x10^9$/L), and in **10-26%**, **intracranial hemorrhage (ICH)** may develop, **up to 80%** of which occurring **prenatally**;



Transfontanelar ultrasound with hyperechogenic intracranial lesions (arrows) suggesting haemorrhage

Silva F, et al. Severe intracranial haemorrhage in neonatal alloimmune thrombocytopenia. BMJ Case Reports 2011; doi:10.1136/bcr.07.2011.4563

Intracranial hemorrhage (ICH)

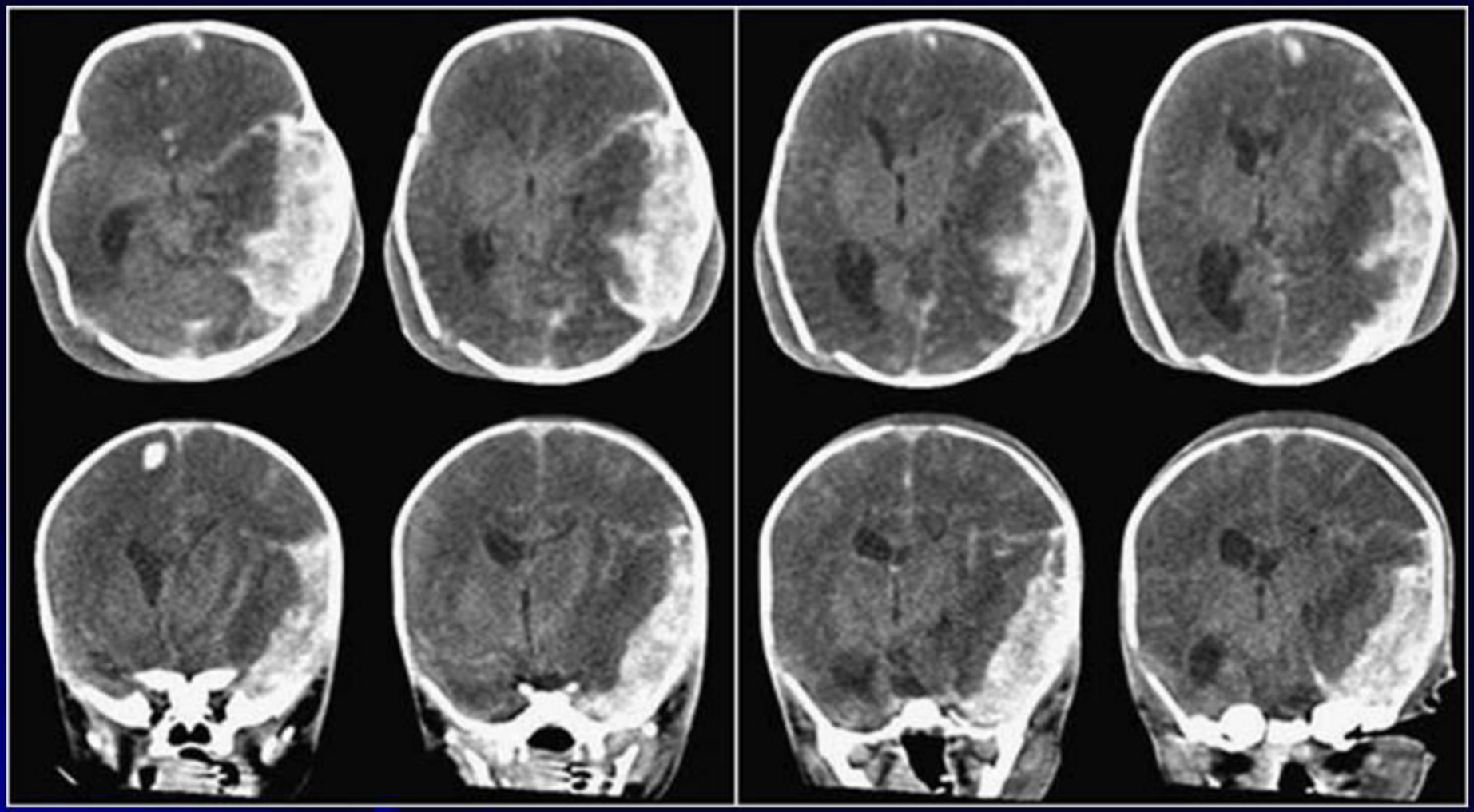


Brain MRI Axial T1WI (A) and T2WI (B): right parietal haemorrhage with blood in early and late subacute stage. Axial T2 gradient echo (C) and sagittal T1WI (D): there are signs of chronic bleeding, with haemosiderin deposits (C) and marked atrophy (D) of the cerebellar hemispheres and vermis.

Silva F, et al. Severe intracranial haemorrhage in neonatal alloimmune thrombocytopenia. BMJ Case Reports 2011; doi:10.1136/bcr.07.2011.4563

Intracranial hemorrhage (ICH)

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



Kato S, et al. Journal of Perinatology (2013) 33, 79–82

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)

Region	Japan	US, Europe, Australia
Methods	MPHA M-MPHA	PIFT MAIPA

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

Japan ～50%
US, Europe 20～70%
] "unresolved" cases the causative Ab not detected;

Smith GA, Ranasinghe E, Ouwehand WH. The importance of using multiple techniques for detection of platelet antibodies. Vox Sang 2007; 93: 306-8

Specificity of HPA Ab in NAIT (US)

Single Ab specificity	Number of cases	(%)	Multiple Ab specificities	Number of cases	(%)
HPA-1a	922	79	HPA-1a+5b	22	2
HPA-1b	43	4	HPA-1b+5b	5	<1
HPA-2b	1	<1	HPA-1b+3a	4	<1
HPA-3a	20	2	HPA-1b+5a	1	<1
HPA-3b	9	<1	HPA-3a+5b	1	<1
HPA-4a	2	<1	HPA-3b+2b	1	<1
HPA-4b	2	<1	HPA-1a+3a+2b	1	<1
HPA-5a	12	1	HPA-1b+3a+5a	1	<1
HPA-5b	109	9	Subtotal	36	<1
HPA-6b	1	<1	Total	1162	
Naka	5	<1			
Subtotal	1126				

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)

Antibody specificity	Number of cases	(%)
HPA-1a	1	<1
HPA-2b	2	2
HPA-3a	17	15
HPA-3b	1	<1
HPA-3a+5b	1	<1
HPA-4a	8	7
HPA-4b	61	52
HPA-5a	1	<1
HPA-5b	12	10
HPA-6b	7	6
HPA-7b	1	<1
Naka	5	4
Total	117	

The risk of ICH was the highest with anti-HPA-3

Manual Standards of Platelet/Granulocyte Antigen, Antibody Testing (in Japanese)

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

Number of pregnancies	Antibody detection rate	Antibody specificity
I	0.19% (19 / 9,750)	4b (4)、5a (2)、5b (13)
II	1.14% (85 / 7,468) *	4b (18)、5b (66)、Nak^a (1)
> III	1.75% (112 / 6,402) **	4b (25)、5a (1)、5b (84)、4b+5b (1)、Nak^a (1)
Unknown	0.69% (7 / 1,010)	4b (2)、5b (5)
Total	0.91% (223 / 24,630)	4b (49)、5a (3)、5b (168)、4b+5b (1)、Nak^a (2)

Ohto H, Miura S, Ariga H, Ishii T, Fujimori K, Morita S et al., Transf Med 2004; 14:399-408

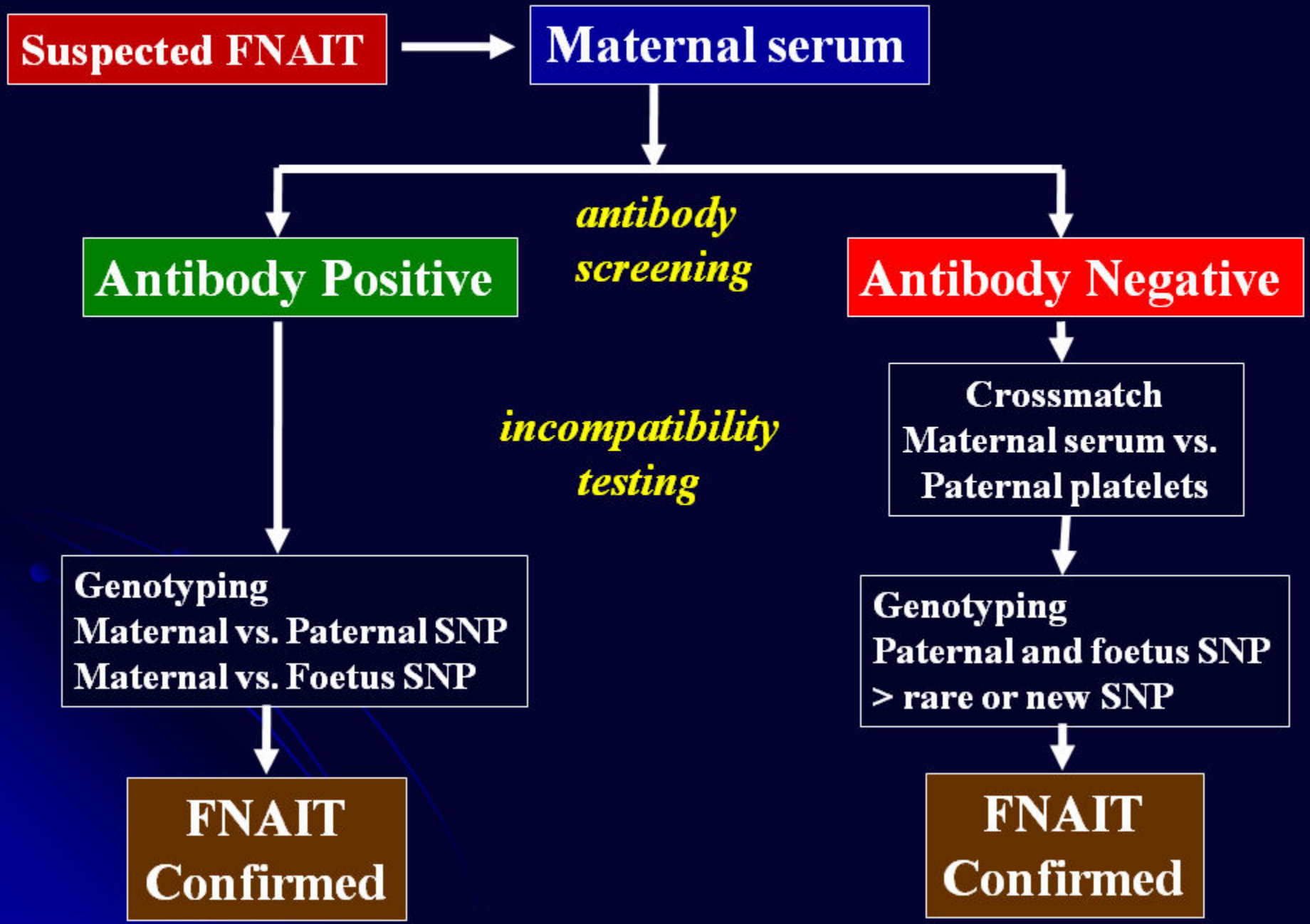
Diagnosis of NAIT

Genotyping for the detection of incompatible SNP

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

Arinsburg SA et al. Am J Hematol 2012; 87: 525-528

Laboratory Diagnostic of FNAIT: Algorithm



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;**

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).

Giers G, Wenzel F, Fischer J, Stockschlader M, Riethmacher R, Lorenz H, Tutschek B. Retrospective comparison of maternal vs. HPA-matched donor platelets for treatment of fetal alloimmune thrombocytopenia. Vox Sanguinis (2010) 98, 423–430 (Düsseldorf, Germany)

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 (<50x10^9/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 30-50x10^9/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;

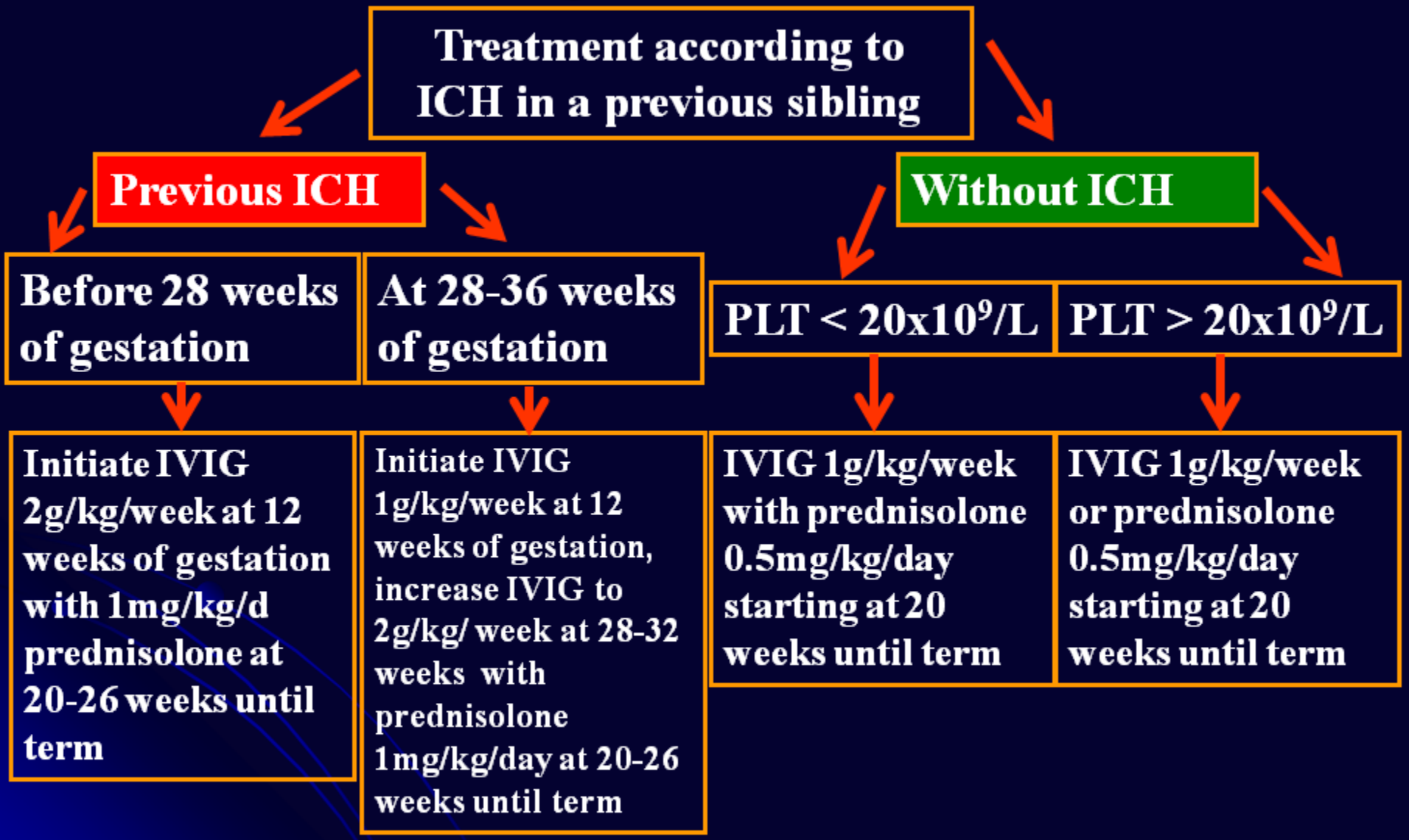
1) **Assess the risk of disease:**

- 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:** ①consider the severity of disease in previously affected sibling; ②test maternal serum for the strength of the anti-HPA antibody (not always predictive);

Non-invasive Management of FNAIT



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

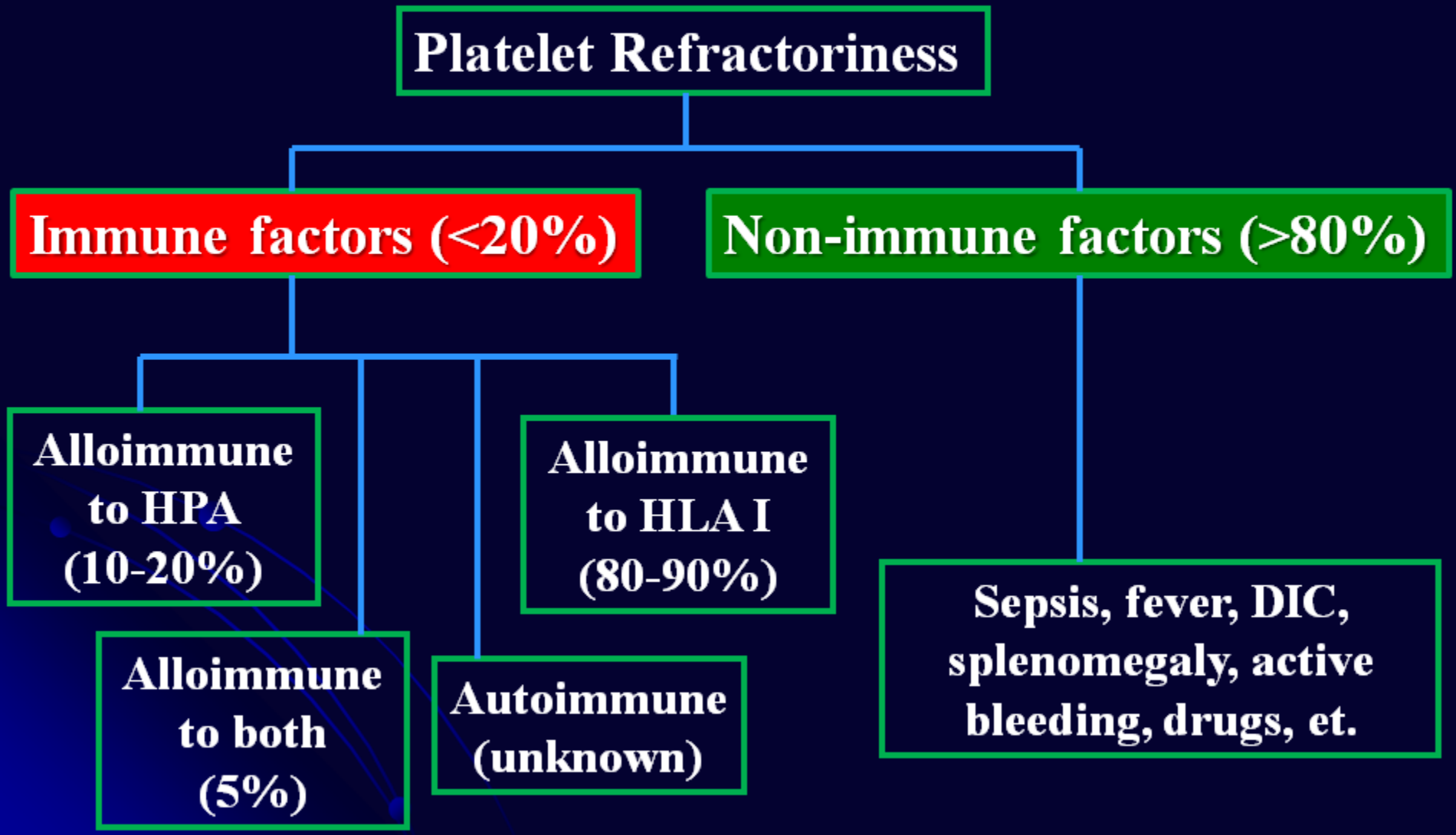
Lack of adequate post-transfusion corrected count increments (CCI) or percent platelet recovery (PPR) following at least 2 transfusions of fresh, random donor ABO-compatible platelets.

⇒ **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



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)

603 Patients	Controls: untreated pooled random donor platelets	Leukoreduced pooled random donor platelets	Leukoreduced single-donor apheresis platelets
Number of Patients	131	137	132
Alloimmunization	45%	18% *	17% *
Refractoriness	16%	7% *	8%
Alloimmunization and refractoriness	13%	3% *	4% *
	* Statistical significance compared to control group		

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

The Trial to Reduce Alloimmunization to Platelets Study Group. N Engl J Med 1997; 337: 1861-1869

Platelet Transfusion Refractoriness (PTR)

Universal prestorage leukoreduction

(late 1990s ~)

Canada Experience

	Pre-universal PSLR group (%)	Post-universal PSLR group (%)	P
Overall refractoriness	27/315 (40)	68/302 (23)	<0.001
Overal alloimmune refractoriness	44/315 (14)	12/302 (4)	<0.001

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

Sefter MD et al. Blood 2004; 103: 333-339

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)

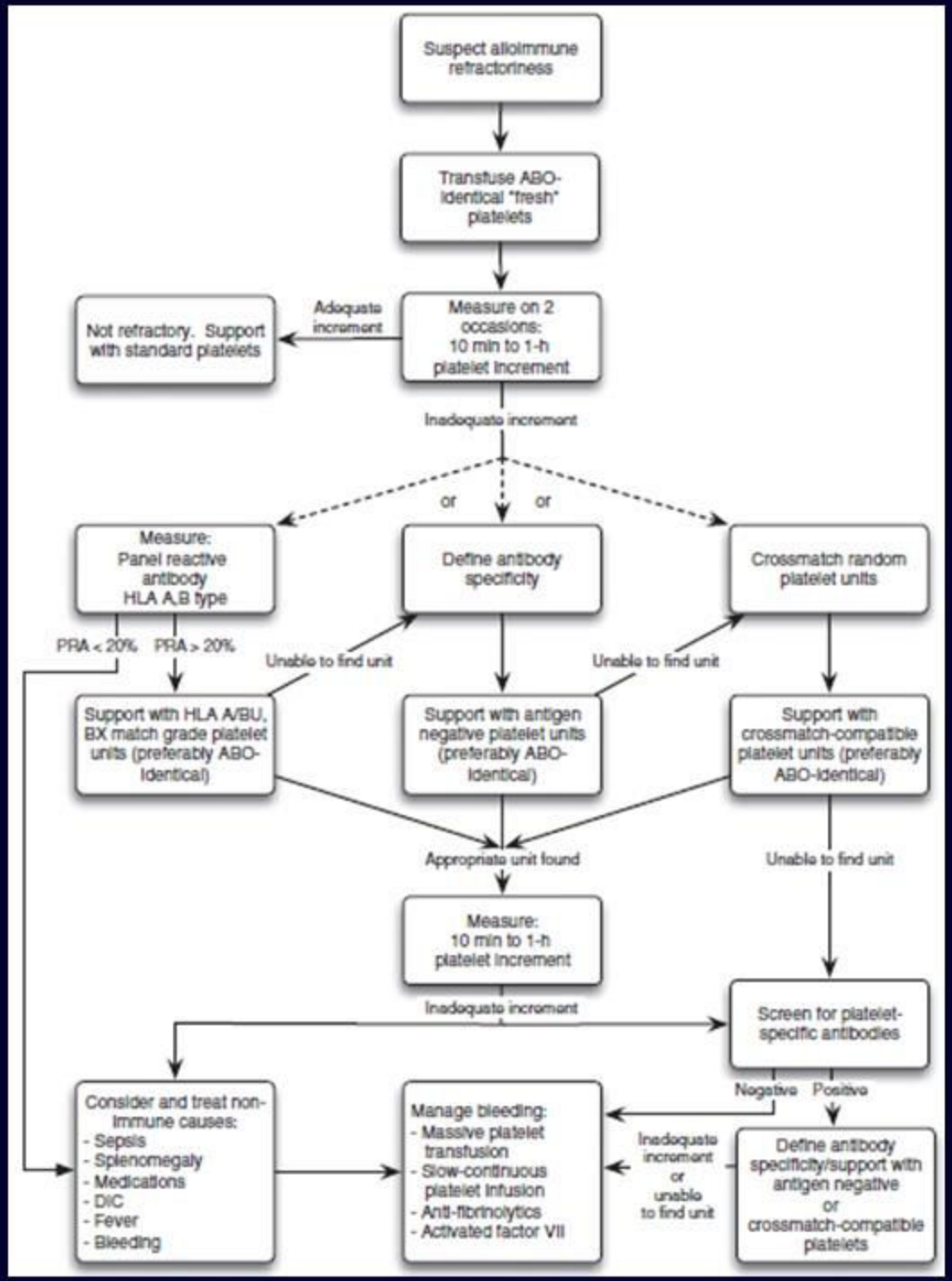
Management

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 (<15x10^9/L), with severe hemorrhagic symptoms, HIT have higher platelet counts (usually >20x10^9/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

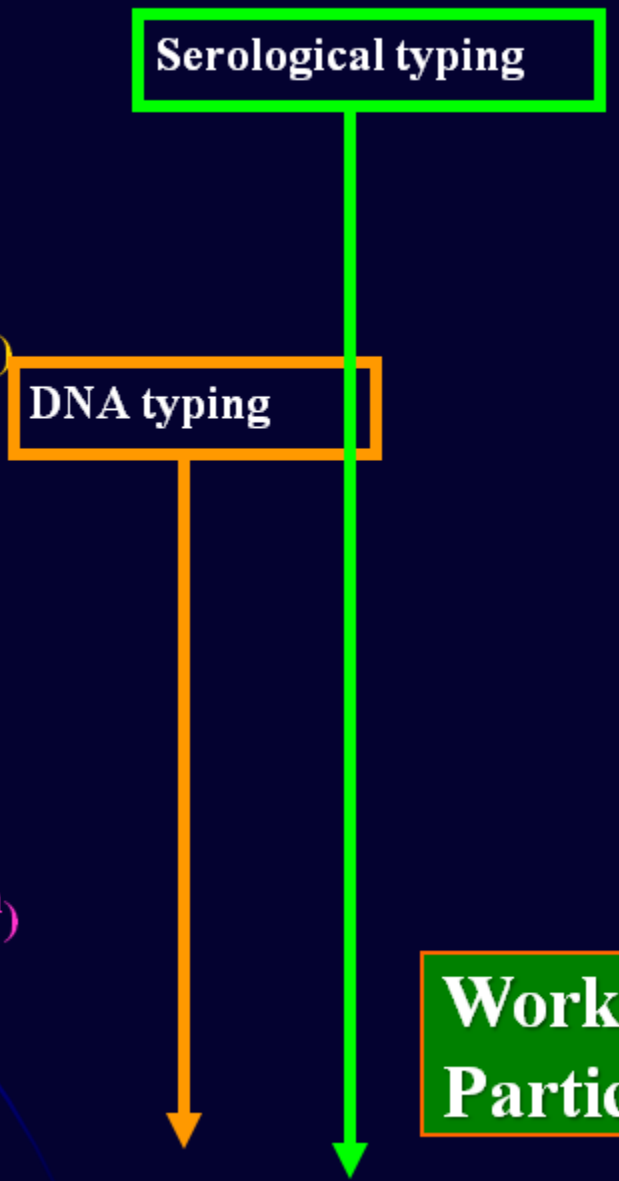
Chair: Dr. Sentot Santoso (Giessen University)
Co-chair: Dr. Nelson H. Tsuno (The University of Tokyo)

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.

International Platelet Immunology Workshop

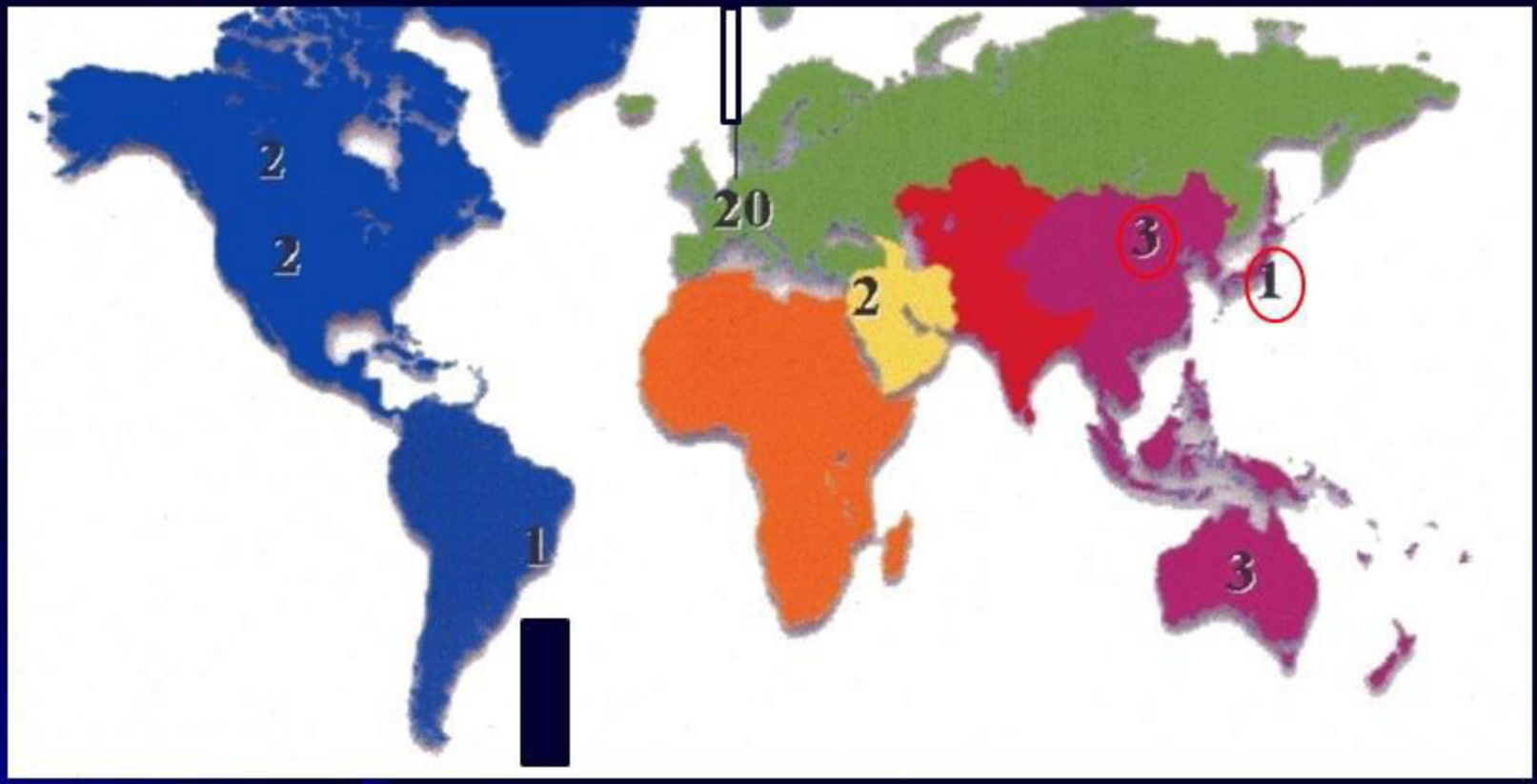
1982	Budapest (1st)
1984	Munich (2nd)
1986	Sydney (3rd)
1988	London (4th)
1990	Los Angeles (5th)
1992	Anaheim (6th)
1994	Amsterdam (7th)
1996	Makuhari (8th)
1998	Oslo (9th)
2000	Vienna (10th)
2002	Vancouver (11th)
2004	Edinburgh (12th)
2006	Cape Town (13th)
2008	Macao (14th)
2010	Berlin (15th)
2012	Cancun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



Workshop in Cancun
Participating labs : 34

ISBT International Workshop – Participant Labs

2012: Among the 34 labs, only 4 (2 Countries) were from Asia



Distribution of HPA alleles among South-Asian and Caucasian

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

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.

Asmarinah A et al. Transf Med 2013; 23: 250-253

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).

1) Curtis BR et al. Transfusion 2002; 42: 1173-1179; 2) Ogata T et al. Transplantation 2005; 79(5): 620; 3) Saw CL et al. Transfusion 2010;50:2638-2642; 4) Nakajima F et al. Vox Sang. 2008; 95(4):318-23; 5) Xu X et al., Thromb Haemost 2013 doi:10.1160/TH13-05-0435

ISBT Platelet Immunobiology WP – Asia Regional Importance

* 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;

ISBT Platelet Immunobiology WP – Asia Regional

Establishment and Activities

* 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;

ISBT Platelet Immunobiology WP – Asia Regional

Establishment and Activities

* **December 1st 2013:** the 2nd 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.

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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)