



Latest Advances in Neonatal Thrombocytopenia

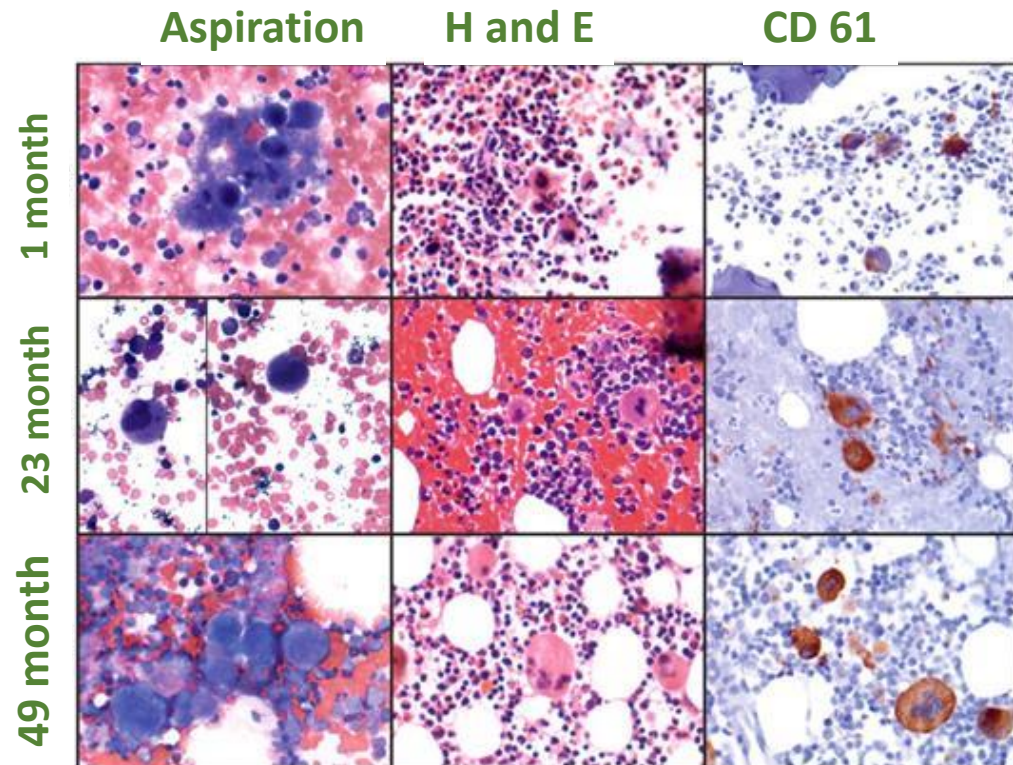
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Outline

- Neonatal thrombocytopenia
 - Prevalence
 - Etiology
 - Common etiologies
 - Management
 - Guideline of platelet transfusion

Physiology of Platelet and Megakaryocyte Development in Neonate

- Platelet in fetus reaching a mean of $150 \times 10^9/L$ by the end of the first trimester



	Neonate	Adults
Tpo concentration	Higher	Lower
Response to Tpo	More sensitive to low Tpo concentration	Less sensitive to low Tpo concentration
MK progenitors	Highly proliferative (10-fold more MKs per progenitor than adults)	Less proliferative than neonatal progenitors
MK size and ploidy	Small size: produce fewer platelets compared to adults	Larger size
	Low ploidy (mostly 2N/4N in vitro)	Higher ploidy levels
MK maturation	Cytoplasmic mature at low ploidy levels	Cytoplasmic maturation increases with ploidy

Platelet Function in Neonate

- Less responsive to agonist
- Enhance primary hemostasis in neonates compared to children and adults
- Decrease platelet adhesion in preterm compared to term neonates

Platelet function (in neonates vs adults)	Primary hemostasis (in neonates vs adults)	Compensatory factors (in neonates vs adults)
↓↓ response to epinephrine	Shorter bleeding times	↑ concentration of VWF
↓ response to thrombin/TRAP	Shorter closure times (PFA-100) ^b	↑ proportion of VWF ultra-large multimers
↓ response to ADP		↑ hematocrit
↓ response to thromboxane		↑ mean corpuscular volume
↓↓ response to collagen		
↓↓ response to rhodocytin ^a		
↓ degranulation		
↑ sensitivity to inhibition by PGE ₁		

Abbreviations: PFA-100: Platelet Function Analyzer-100; PGE₁, prostaglandin E₁; TRAP, thrombin receptor activating peptide; VWF, von Willebrand factor

^a CLEC-2 ligand

^b Closure times in response to collagen/epinephrine and collagen/ADP

Prevalence of Neonatal Thrombocytopenia

- Defined as a platelet count below $150 \times 10^9/L$ in newborns
- Occurs in approximately 1–5% of all neonates,
- NICUs are range from 22% to 35%, with increased rates observed in those with lower gestational ages.
- Classification of severity
 - Mild $100-150 \times 10^9/L$
 - Moderate $50-100 \times 10^9/L$
 - Severe $<50 \times 10^9/L$



Etiology of Neonatal Thrombocytopenia

- **Intrauterine onset**

- Immune thrombocytopenia
- Intrauterine infection
- Chromosomal abnormality

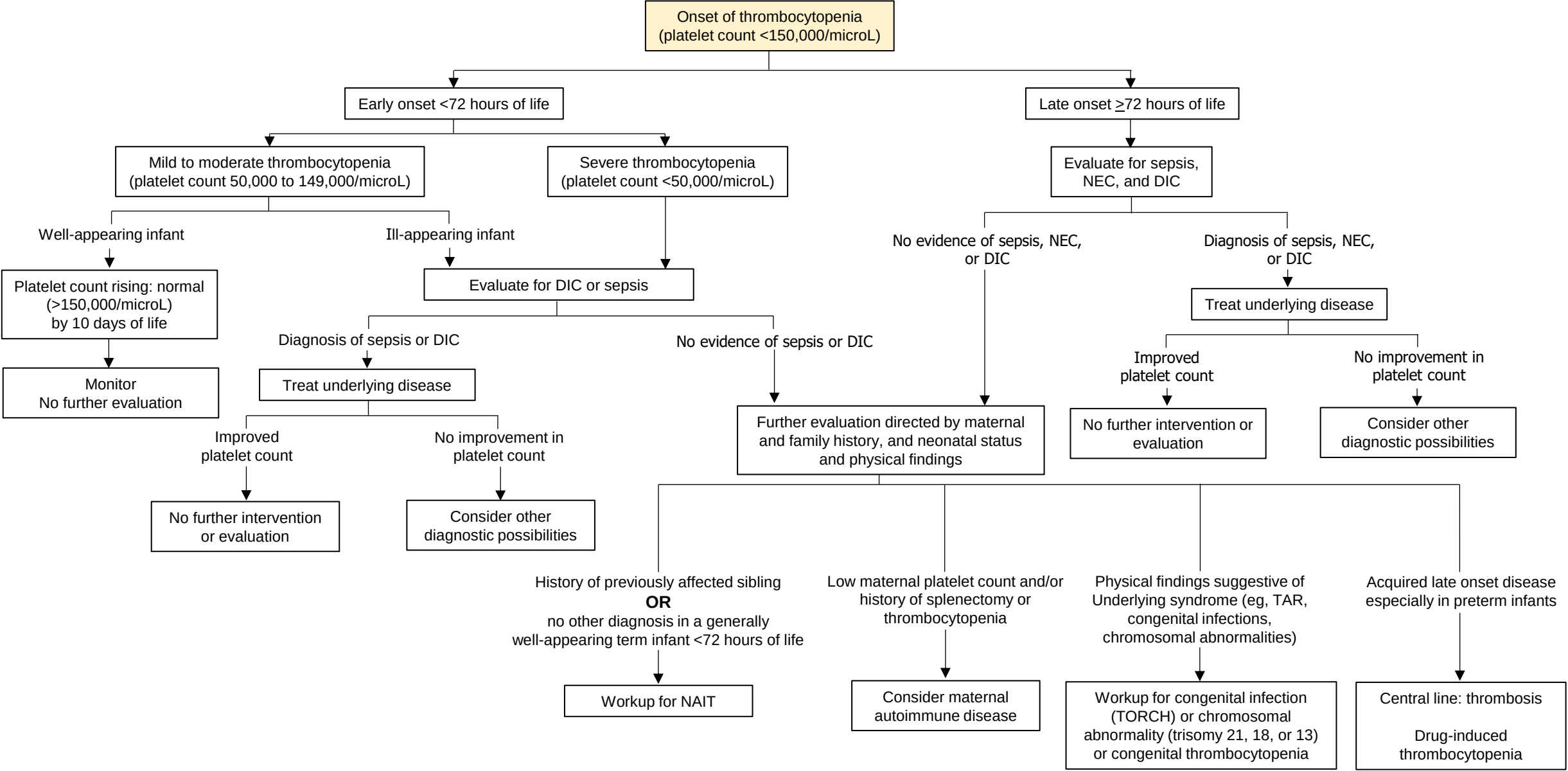
- **Early onset (<72 h)**

- Placental insufficiency
- Perinatal asphyxia
- Perinatal infection
- Immune thrombocytopenia
- Disseminated intravascular coagulation
- Congenital thrombocytopenia

- **Late onset (>72 h)**

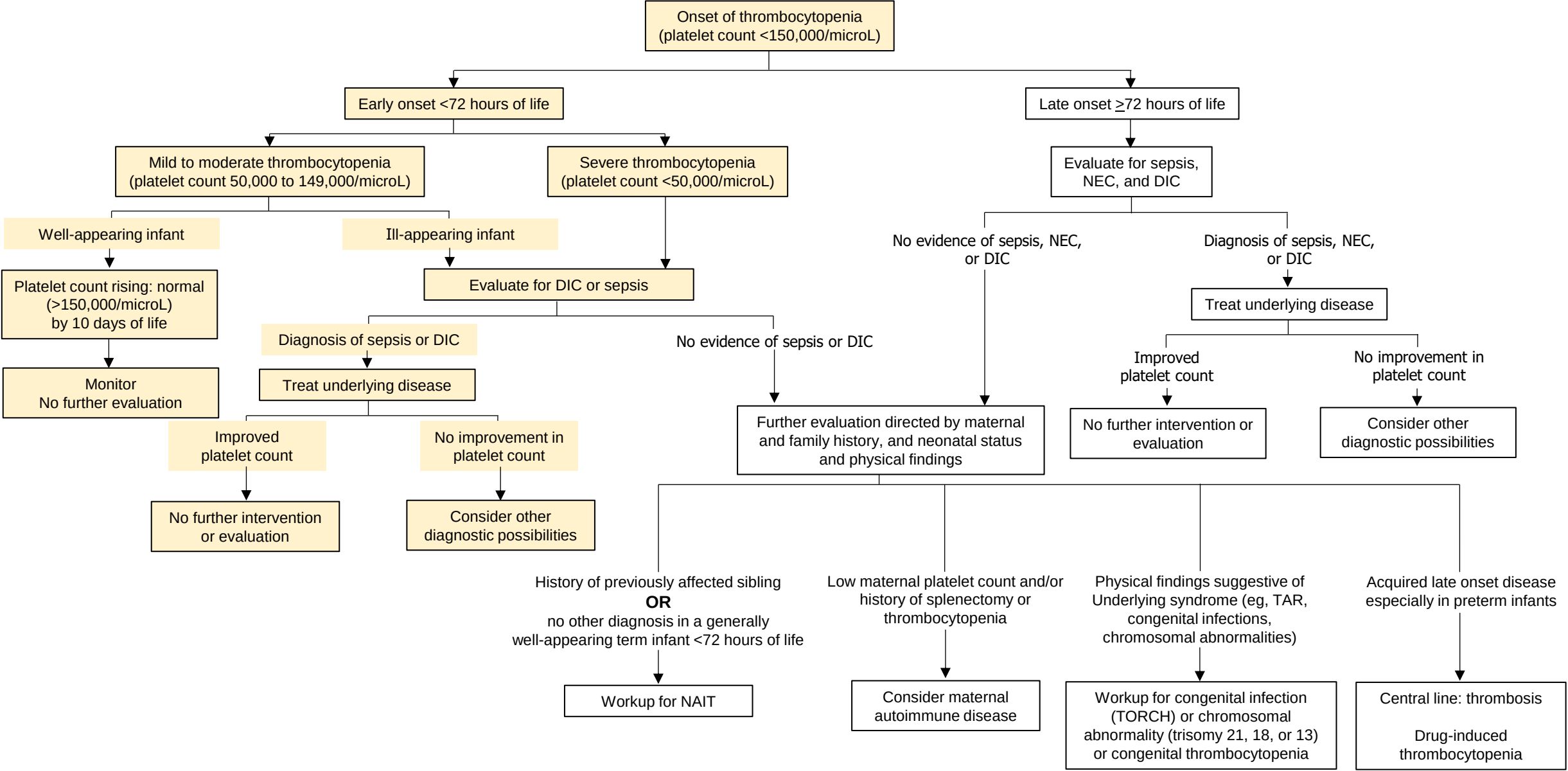
- Late neonatal sepsis
- Necrotizing enterocolitis
- Amegakaryothrombocytopenia
- Giant hematoma
- Congenital thrombocytopenia

Diagnostic Evaluation of Neonatal Thrombocytopenia



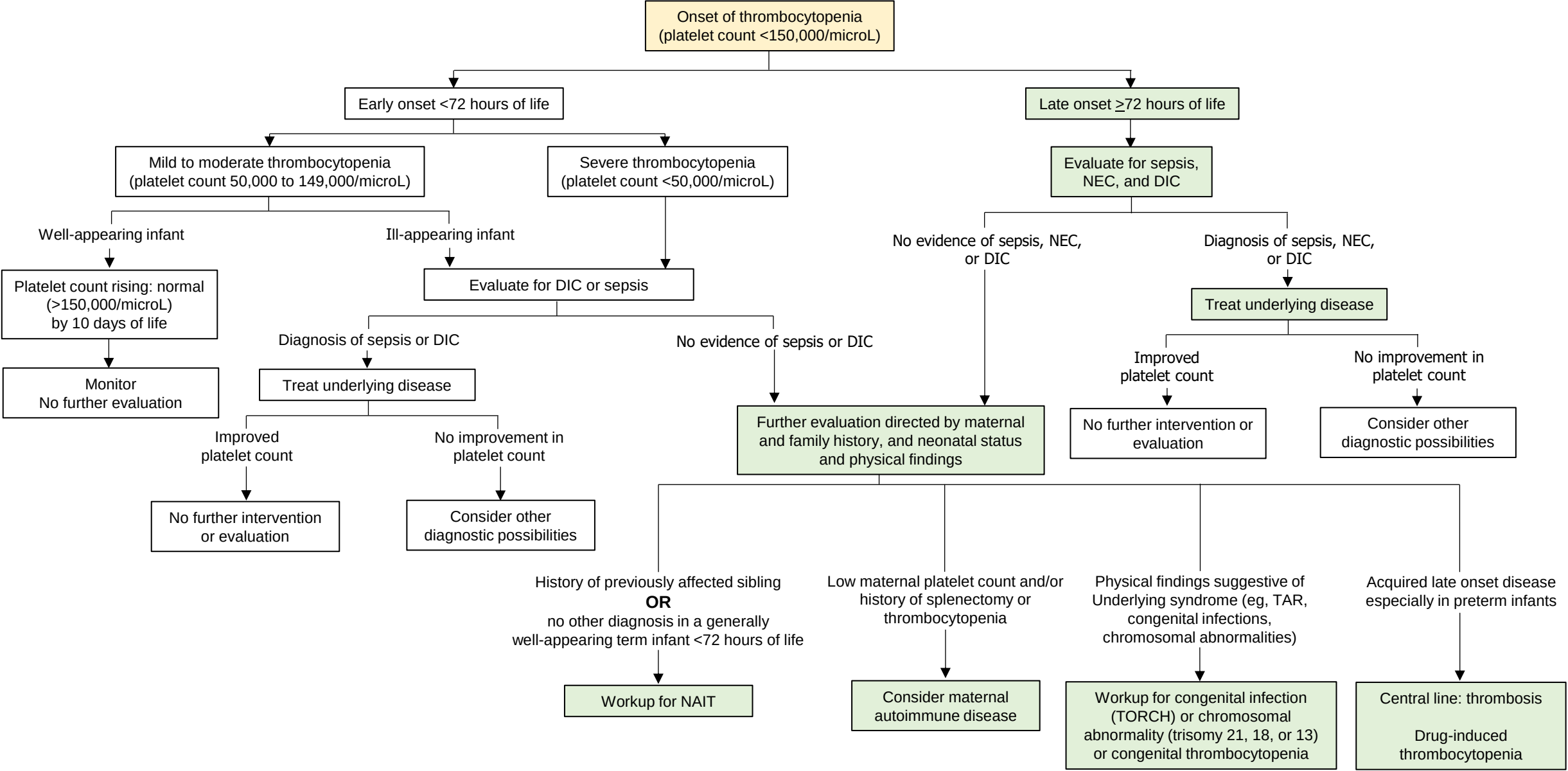
NEC, necrotizing enterocolitis; DIC, disseminated intravascular coagulation; TAR, thrombocytopenia-absent radius syndrome; NAIT, neonatal alloimmune thrombocytopenia; TORCH, toxoplasmosis, other (syphilis), rubella, cytomegalovirus, herpes simplex virus.

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NEC, necrotizing enterocolitis; DIC, disseminated intravascular coagulation; TAR, thrombocytopenia-absent radius syndrome; NAIT, neonatal alloimmune thrombocytopenia; TORCH, toxoplasmosis, other (syphilis), rubella, cytomegalovirus, herpes simplex virus.

Diagnostic Evaluation of Neonatal Thrombocytopenia

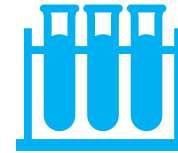


NEC, necrotizing enterocolitis; DIC, disseminated intravascular coagulation; TAR, thrombocytopenia-absent radius syndrome; NAIT, neonatal alloimmune thrombocytopenia; TORCH, toxoplasmosis, other (syphilis), rubella, cytomegalovirus, herpes simplex virus.

Investigation



- CBC, peripheral blood smear
- MPV, Immature platelet fraction
- Bone marrow study



- Blood culture, TORCH study
- Flow cytometry
- Antibody screening/identification



- Cranial ultrasound



- Chromosomal testing
- Genetic of congenital thrombocytopenia

Case Study

Patient profile: A 38⁺³ weeks, Apgar 9, 10

Mother has no underlying disease

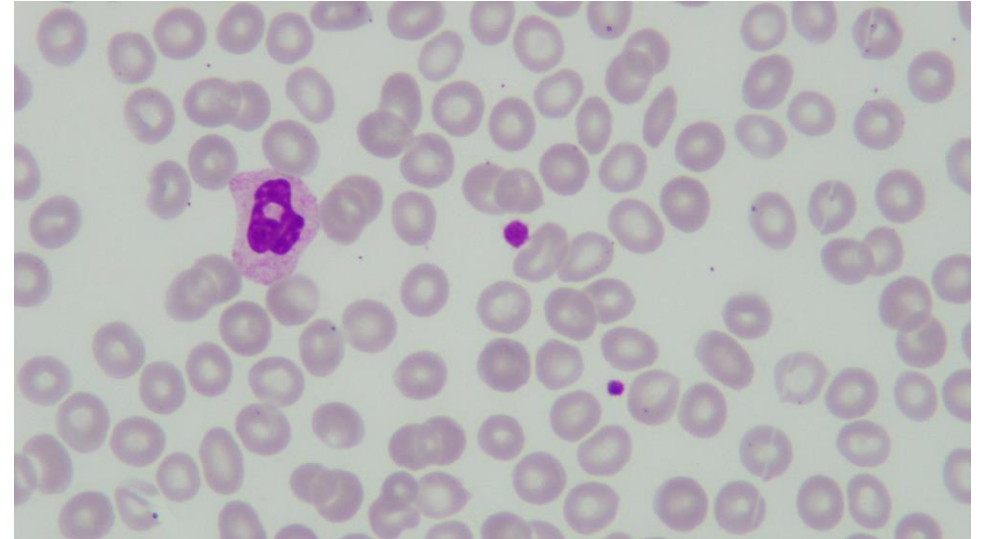
Patient history:

- At age 2 days old developed petechial hemorrhage CBC showed Hb 17 g/dL, WBC 17,960/ uL, PMN 65%, neutrophil 23% platelet 6,000/uL, workup TORCH negative. The patient was treated with IVIG 1 g/kg without improvement

PE: T 37.2 °C, Pulse 151/min, RR 54/min, BP 80/37 mmHg

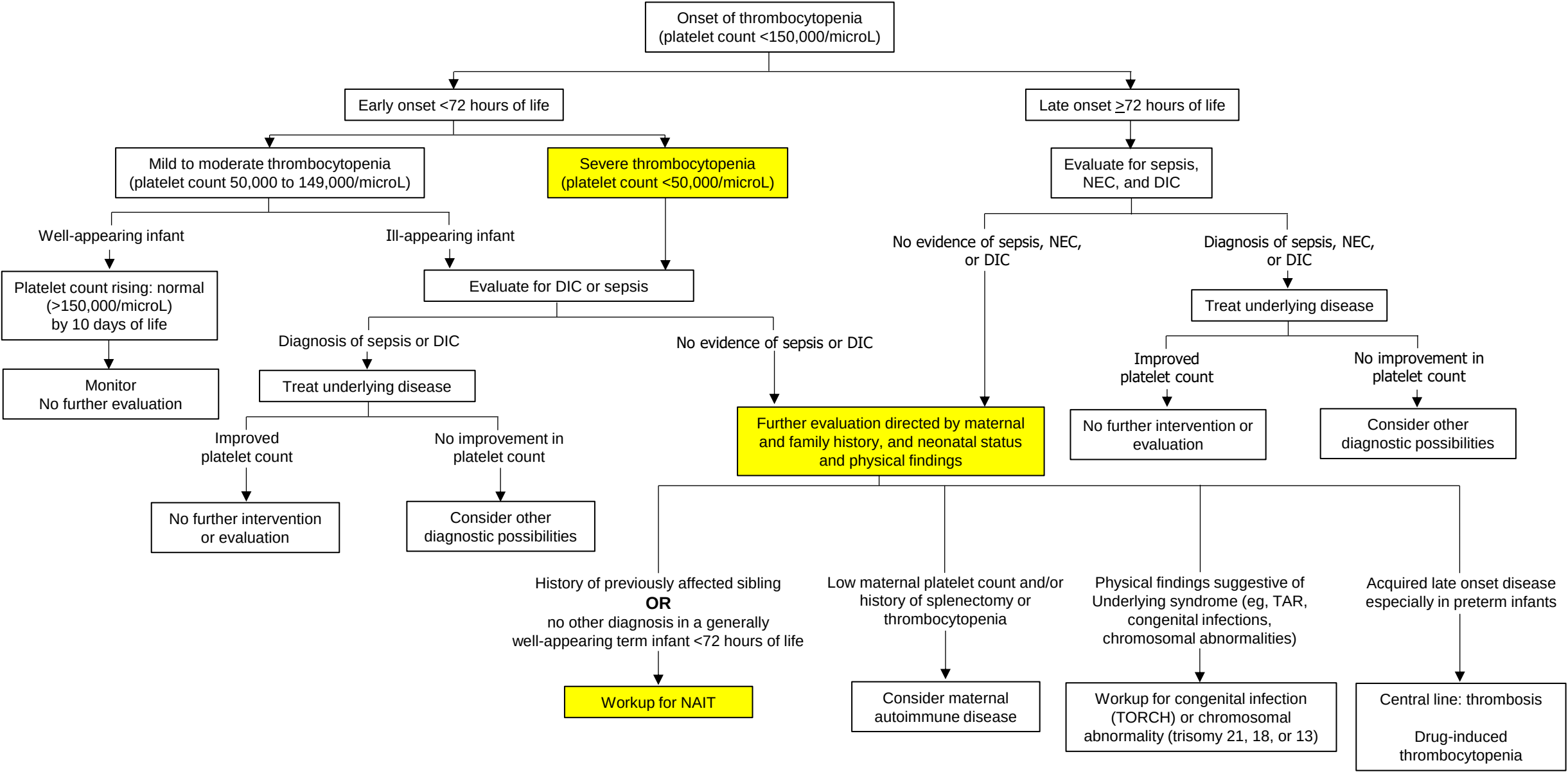
AF 2x2 cm, HC 33.5 cm

Purpura at shin, petechial hemorrhage



Problem: Neonatal thrombocytopenia

Diagnostic Evaluation of Neonatal Thrombocytopenia



NEC, necrotizing enterocolitis; DIC, disseminated intravascular coagulation; TAR, thrombocytopenia-absent radius syndrome; NAIT, neonatal alloimmune thrombocytopenia; TORCH, toxoplasmosis, other (syphilis), rubella, cytomegalovirus, herpes simplex virus.

Investigation

- Platelet screening antibody (MAIPA)

HLA antibody: **detected**

- Platelet immunofluorescent test (PIFT): not detected
- Anti HPA3a negative

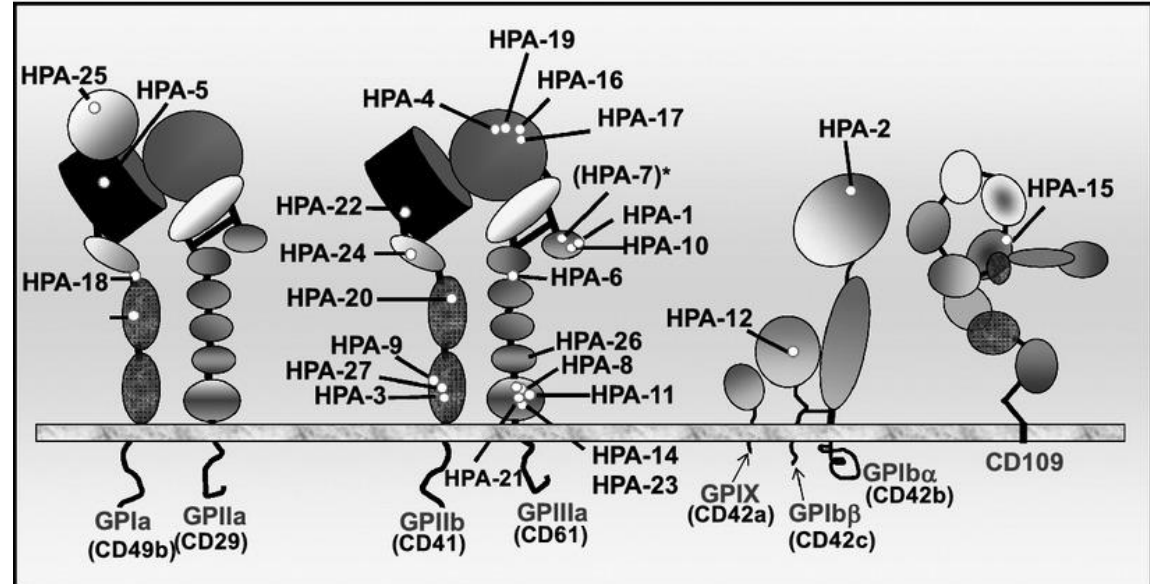
HLA	HLA-A	HLA-A	HLA-B	HLA:B
Mother	A*11	A*24	B*46	B*51
Father	A*68	A*24	B*13	B*65
Patient	A*24	A*24	B*46	B*65

HPA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	21
Mother	ab	aa	bb	aa	aa	aa	aa	aa	aa	aa	a	NT	aa	aa	aa	NT	aa	aa
Father	aa	aa	aa	aa		aa	aa	aa	aa	aa	aa	NT	aa	aa	aa	NT	aa	aa
Patient	aa	aa	ab	aa	aa	aa	aa	aa	aa	aa	aa	NT	aa	aa	aa	NT	aa	aa

Diagnosis: Neonatal alloimmune thrombocytopenia (HLA antibody)

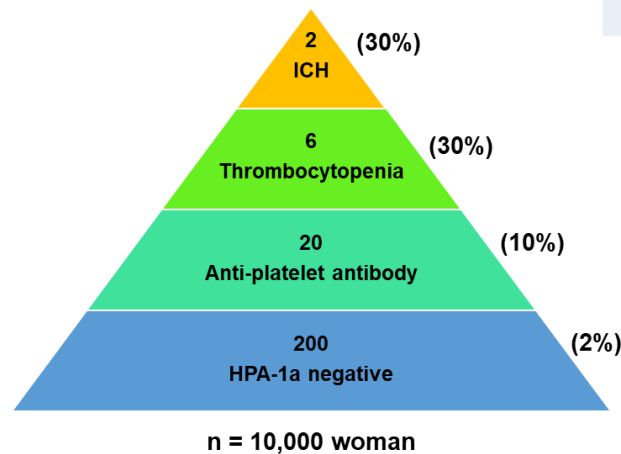
Platelet Antigen

- Human platelet antigen: more than 25 antigens
- Human leukocyte antigen (HLA class I)
- Blood group antigen



Fetal and Neonatal Alloimmune Thrombocytopenia

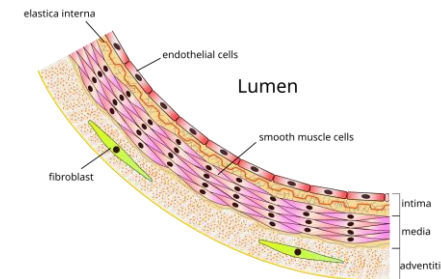
- Definition: maternal antibodies directed against fetal platelet antigens inherited from the father
- 15% with platelet $<50 \times 10^9/L$ develop ICH
- High recurrence rate for the subsequent pregnancy (75-100%)



	N	Incidence
Burrows and Kelton, 1993	15,932	1 in 1,700
Uhrynowska, 2000	4,101	1 in 2,400
Turner, 2005	26,000	1 in 5,000
Kjeldsen-Kragh, 2007	100,448	1 in 1,700

Anti-HPA-1a
 Suppress of megakaryocytopoiesis
 Destroy endothelial cell: endothelial express antigen to the specific antibody ($\alpha V\beta 3$)

	Caucasian	Asian
Common antibody	HPA-1a	HPA-5b, HPA-4b
Report association which increase antibody production	MHC class II allele HLA-DRB3*01:01	NA
Risk of antibody formation in pregnant women	9.7-12.7%	
Intracranial hemorrhage	10-25%	
Alloantibody destroy endothelium	+	



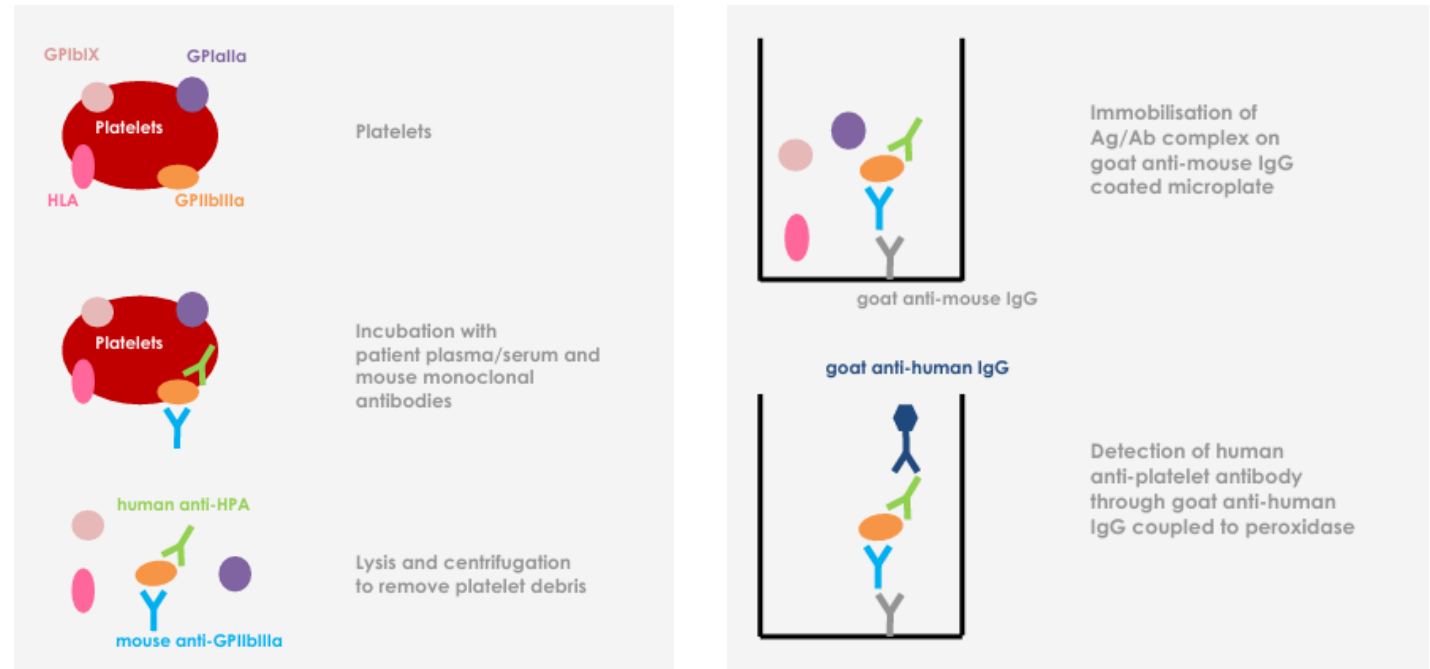
de Vos TW, et al. Transfus Apher Sci. 2020;59:102704.
 Constantinescu S, et al. Maedica (Bucur). 2012;7:372-6.
 Santoso S, et al Arterioscler Thromb Vasc Biol. 2016;36:1517-24.

Laboratory Diagnosis of Platelet Antibody and Limitation

Test	Description	Used / Advantages	Limitations
I. Indirect platelet Ab test	Identify platelet-specific Ab in patient serum or plasma	Used for diagnosis of NAIT, PTP, & MPTR ^a	- Does not differentiate alloantibodies and autoantibodies - Not recommended for ITP (helpful in some cases)
a. ELISA	- Uses captured platelet GPs from lysed platelets as source of known HPAs - Multiple methodologies (ACE, MACE, MAIPA) for GP capture	- GP-specific (specific for antibodies to HPAs) - No reactivity from non-HPA Ab (HLA, blood group) - Preferred for Ab identification in NAIT (using maternal serum)	- Results reported qualitatively - Clinical utility of Ab titer is controversial - Commercial kits detect only common platelet Ab - Low titer and low avidity Ab may not be detected
b. Flow cytometry	Uses intact platelets	- Detects some Ab that cannot be detected by GP-specific tests - More sensitive for labile and low-density antigens	- Less specific for Ab to HPAs - May detect HLA, blood group Ab - Does not identify specific Ab target
II. Direct platelet Ab tests	Detect autoantibodies attached to patient platelets	More sensitive and specific than indirect tests for autoantibody detection	- Sensitivity is compromised by severe thrombocytopenia - Not recommended for ITP (helpful in some cases)
a. ELISA	Detects GP-specific Ab by eluting bound Ab from patient platelets or using solubilized patient platelets with bound autoantibodies	Very specific	Low sensitivity (50%)
b. Flow cytometry	Detects any platelet associated Ab	Very sensitive	Low specificity, often positive in non-ITP
^a NAIT, neonatal alloimmune thrombocytopenia, PTP, post-transfusion purpura, MPTR, multiple platelet transfusion refractoriness, ITP, immune thrombocytopenia; HPA, human platelet antigen; ACE, antigen capture ELISA; MACE, modified ACE; MAIPA, monoclonal antibody immobilization of platelet antigens. The results of antibody testing shown in this table can be further supported by identifying what antigen(s) are on the patient's platelets by phenotyping or genotyping methods (see text).			

Monoclonal Antibody-specific Immobilization of Platelet Antigen (MAIPA)

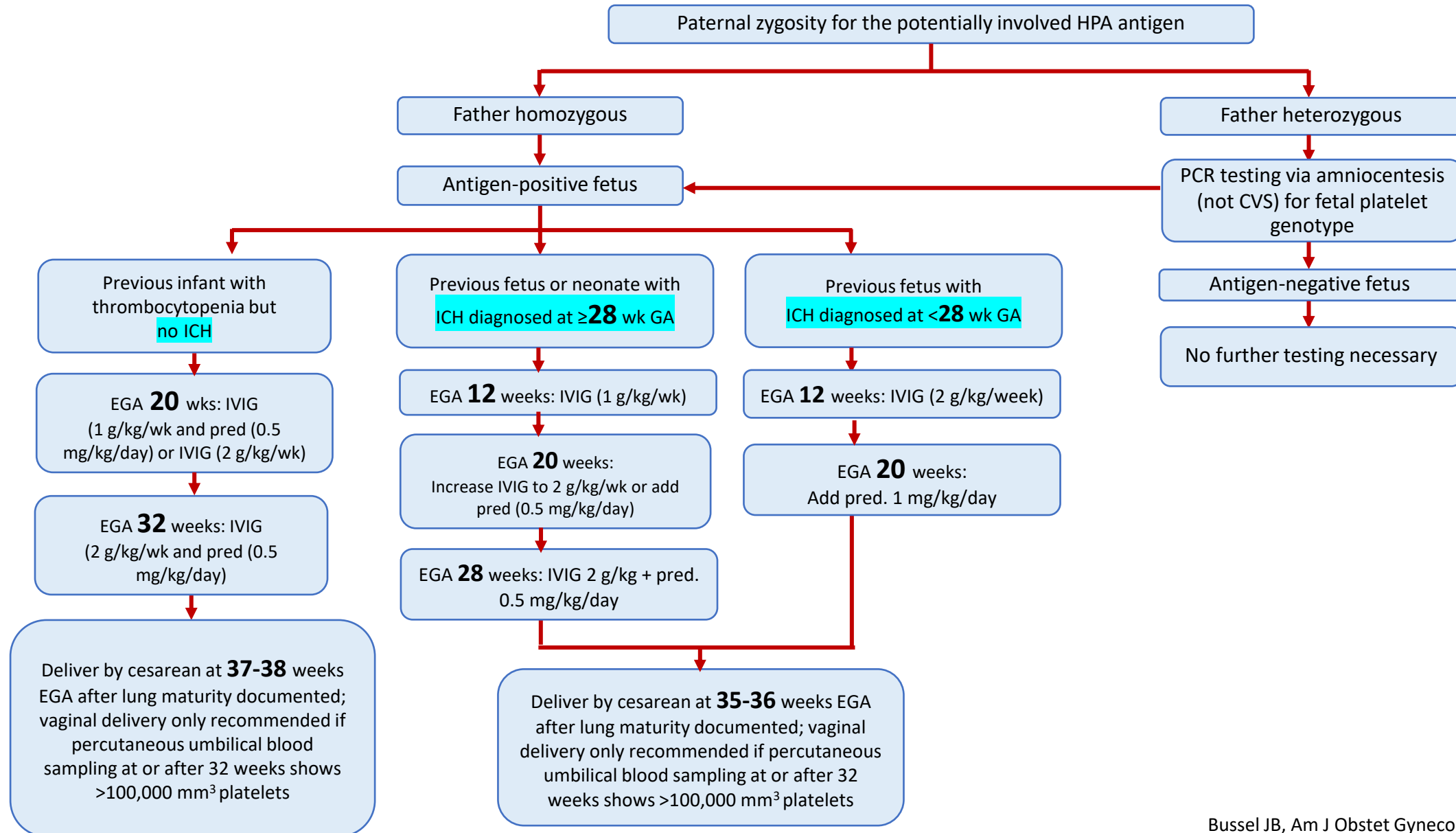
- Qualitative technique for platelet antibody detection and/or identification
- It is considered to be the gold standard method in the platelet immunology field



Management of Fetal and Neonatal Alloimmune Thrombocytopenia

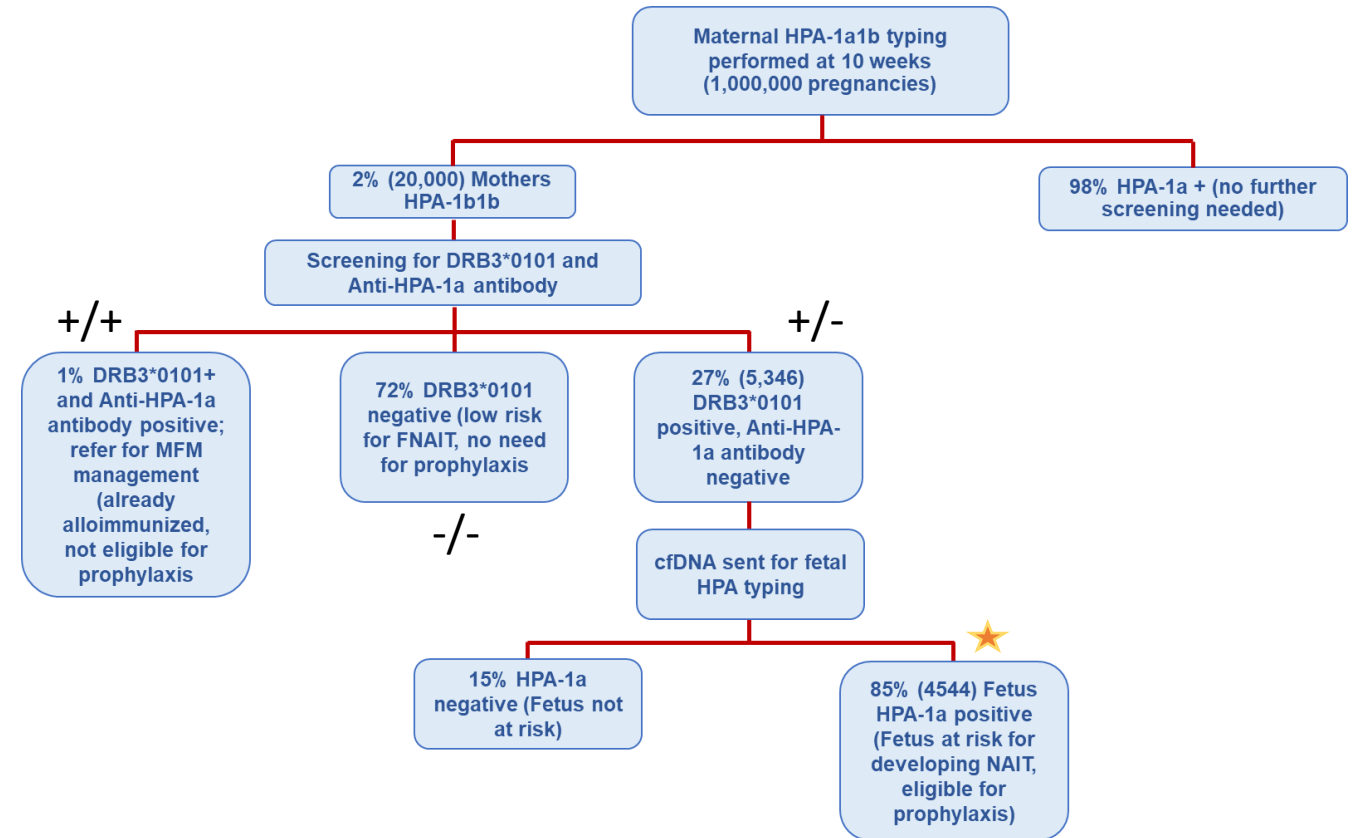
Recommendation	Indication/Treatment	Note	Level of evidence/recommendation
Postnatal			
Cranial ultrasound	Within first 24 h after birth		Very low, strong
Platelet transfusion	< 30,000/uL	HPA matched platelet or random platelet	Moderate, strong
IVIG	1 g/Kg (1-3 days)		
	Methylpredisolone 1 mg iv every 8 h*		
Antenatal			
	Risk classification; Standard risk: No ICH in sibling, high risk: Prior sibling had ICH> 28 weeks, very high-risk ICH< 28 weeks		
Women or sister of women with previous history of FNAIT	Refer to the fetal medicine	Fetal HPA typing (non-invasive method)	Very low, strong
Antibody titer	Identify neonate at risk		Low, weak
HLA DRB3*01:01 of HPA 1b/1b women	Identify neonate at risk		
IVIG	-1 g/kg/wk12-16 wk GA then 2 g/kg/wk at 20 wk -1 g/kg/wk at 12-16 wks, add steroid 1 mg/kg/day at 20 wk -0.5 g/kg at 12-16 wks for the entire pregnancy -2g/kg/wk at 12-16 week for entire pregnancy -2 g/kg/wk at 12-16 week, then steroid 1 mg/kg/day at 20 wk		
Delivery			
Delivery at term	Cord blood CBC, Prepare HPA selected platelet		Low, strong

Management of Next Pregnancy



Novel Approach, Prophylaxis and Therapy in Neonatal HPA-1-a Alloimmune Thrombocytopenia

- **Investigation:** cell-free fetal DNA for HPA genotype
- **Prophylaxis:** NAITgam: Hyperimmune anti-HPA-1a IgG
- **Treatment:** neonatal Fc receptor (FcRn receptor) blockage (Nipocalimab) → lower IgG levels



Frequencies of Human Platelet Antigens in Northeastern Thailand and in Other Populations

Antigens	Other names	Thais	Northeastern Thais	Japanese*	Taiwan Chinese ²	Koreans ³	Indonesian ⁴	Whites ⁵	South American Indians ⁶
HPA-1a	PI ^{A1} , Zw ^a	98.5	100.0	>99.9	100.0	100.0	>99.40	97.9	>99.9
HPA-2b	Koa, Sib ^a	4.8	15.9	25.4	9.0	NA	NA	14.6	NA
HPA-3a	Baka, Lk ^a	56.0	60.2	78.9	77.0	87.3	72.89	87.7	89.3
HPA-4a	Yukb, Pen ^a	100	98.8	99.9	100.0	100.0	>99.40	99.9	>99.9
HPA-4b	Yuka, Pen ^b	0	1.9	1.7	0.5	1.6	0.59	0.2	0.9
HPA-5b	Bra, Zav ^a	3.2	5.4	10.8	NA	NA	9.26	20.6	4.9
Nak ^a			97.7	93.5	96.0	NA	95.83	NA	NA

*Japanese Workshop data, provided by Y. Shibata (written communication, June 1994).

NA, Not available

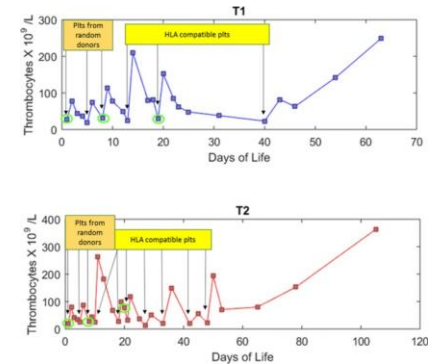
Urwijitaroon Y, Transfusion. 1995;35:868-70.
Kupatawintu P, et al. Immunohematology. 2005;21:5-9.

Report Case of Other Platelet Antigen-Related Neonatal Alloimmune Thrombocytopenia

- A female neonate platelet count of 15,000 /uL
- Bilat cephalhematoma and upper GI bleeding
- Positive anti-NaK^a (located in GPIV or CD36)
- Treatment: hydrocortisone, IVIG, platelet

Report Cases of HLA Related Neonatal Alloimmune Thrombocytopenia

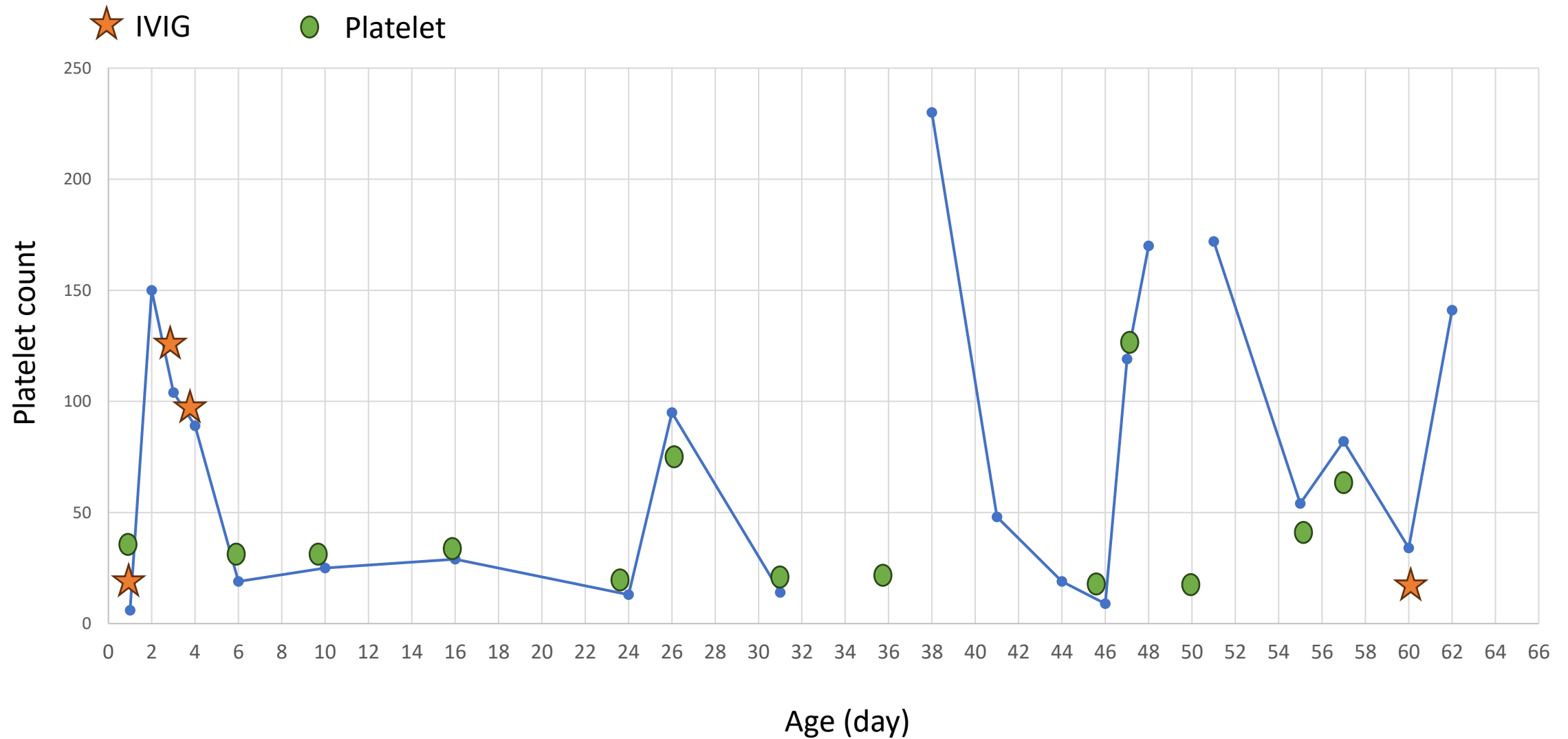
- 37 weeks female twins born with platelet count 28,000 and 20,000/uL
- Maternal screening: no anti-HPA antibodies detected
- Luminex single antigen on D8 showed a potent **HLA antibody (HLA-B57, B58)**
- MRI brain: 8 mm cerebral bleeding in 2nd twin
- Treatment: IVIG and HLA matched platelet



- 39 weeks D5 female neonate platelet count 63,500 /uL then 36,800/uL
- Maternal screening: no anti-HPA antibodies detected
- HLA antibody to **HLA class I (HLA-A11)**
- Cranial ultrasound: no bleeding
- Treatment: IVIG

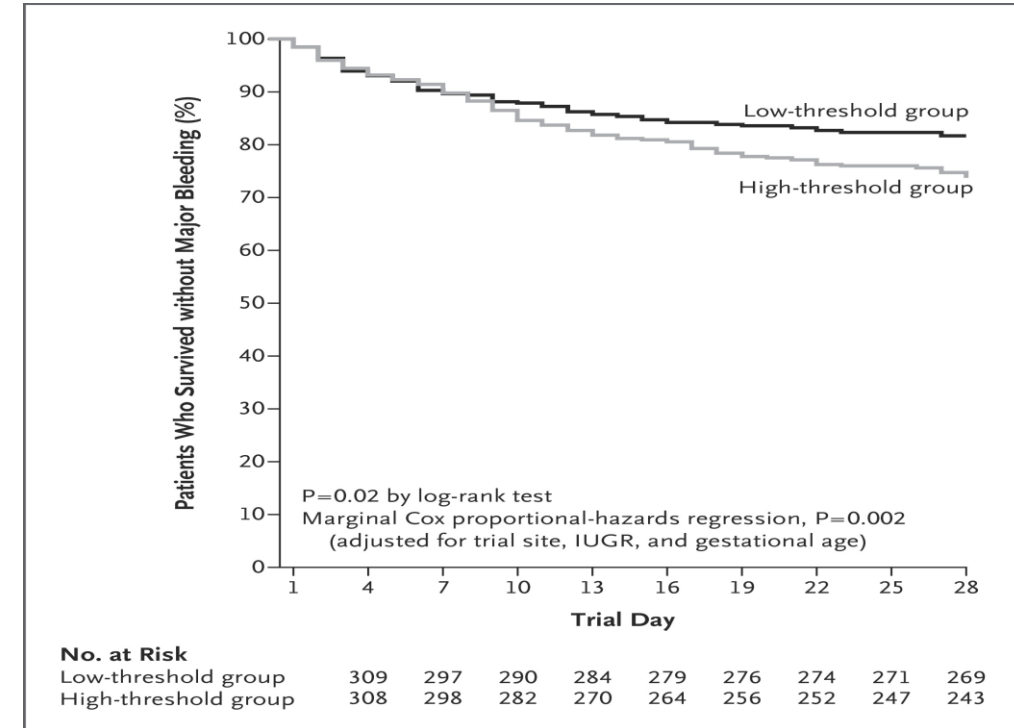
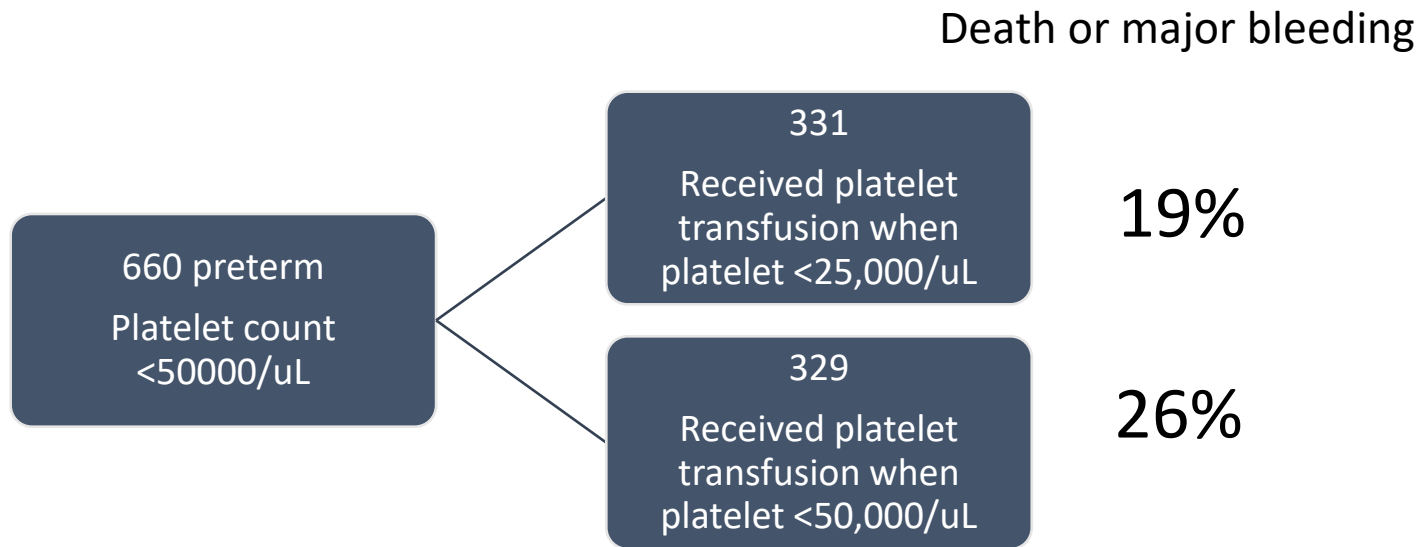
Report from Thai Red Cross Society in 34 neonates
HLA positive 50%

Treatment



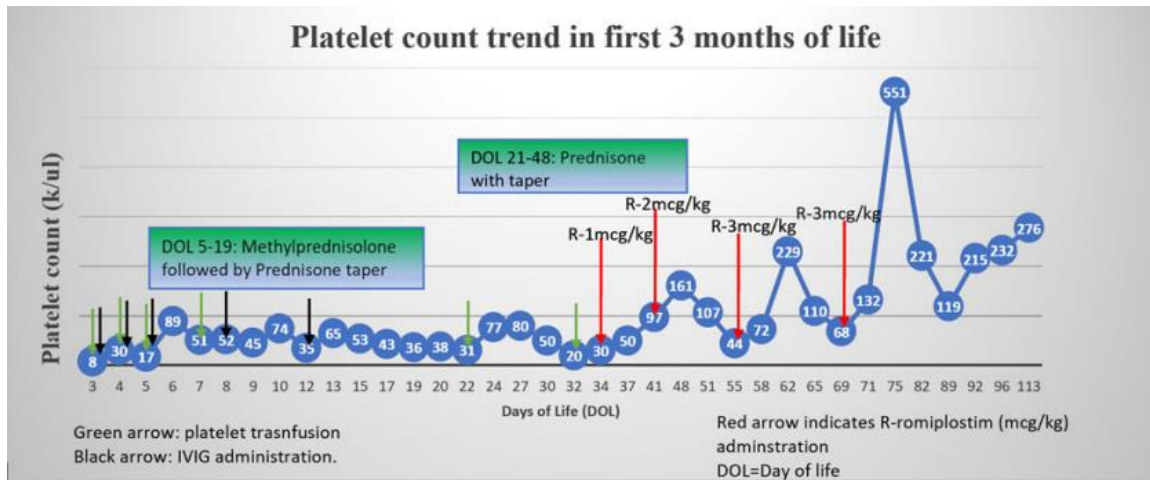
Platelet Transfusion Threshold in Neonate

- A RCT included preterm <34 weeks

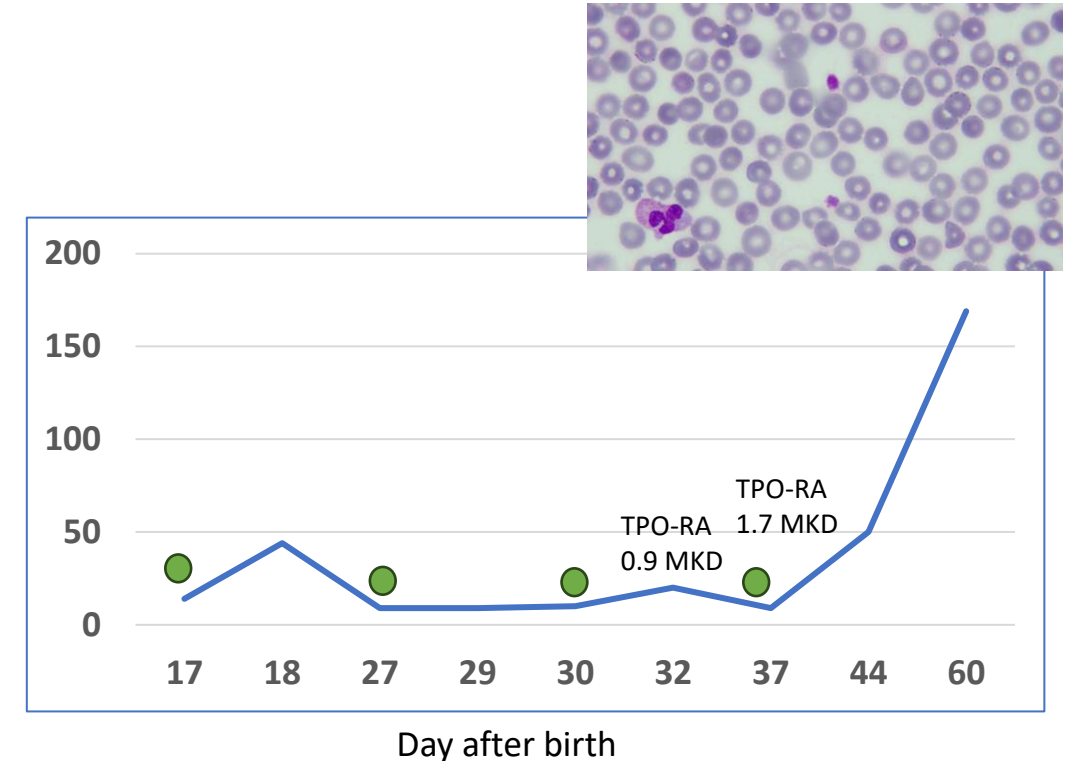


Curley A, et al. Randomized Trial of Platelet-Transfusion Thresholds in Neonates. N Engl J Med. 2019;380:242-251.

Report of Thrombopoietin Receptor Agonist in Neonate with Immune Thrombocytopenia



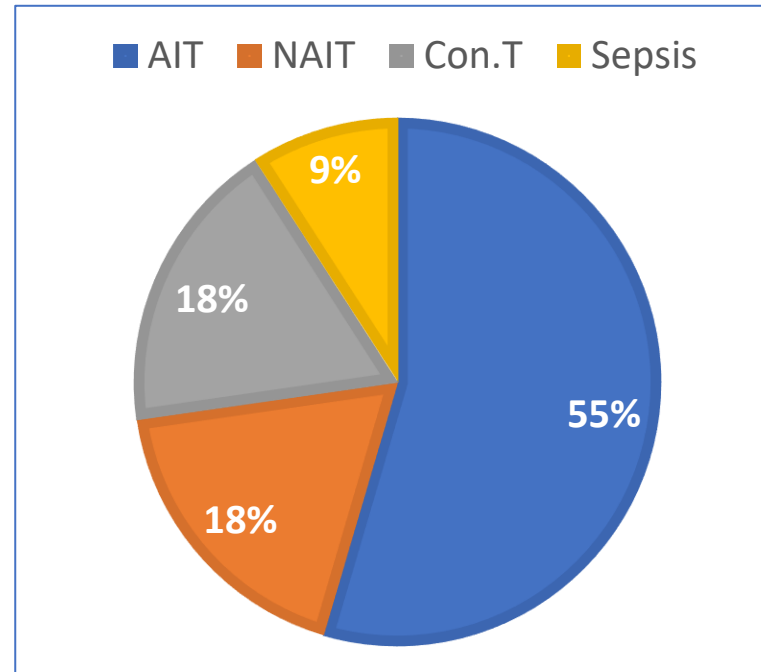
A full-term female born with the mother had history of immune thrombocytopenia (ITP) received several platelet transfusion and successful increase platelet count after TPORA



A full-term neonate with thrombocytopenia suspected Immune thrombocytopenia (Allo or auto)

Neonatal Thrombocytopenia

- Consult during 3 years = 11 neonates
- Excluded infection, TORCH
- Median age 3 (1-29) days
- Median platelet count 40,500 (10,000-95,000)/uL
- Median time to recovery 1.8 (0.4-5.7) month
- No serious bleeding



Treatment

- IVIG total dose of 2 g/kg and platelet transfusion (5)
- IVIG + corticosteroid (1)
- Corticosteroid (1)

Congenital Thrombocytopenia in Neonatal Thrombocytopenia

- **Persistent or Unexplained Thrombocytopenia**

- Platelet count remains **low beyond 1–2 weeks** of life
- No clear evidence of infection, DIC, maternal ITP, or alloimmune thrombocytopenia (NAIT)

- **Abnormal Platelet Size or Morphology**

- **Small platelets:** Wiskott-Aldrich Syndrome (WAS)
- **Large platelets:** Bernard-Soulier Syndrome (BSS), MYH9-related disorders (May-Hegglin Anomaly)
- **Giant platelets:** Paris-Trousseau Syndrome(**with absent α -granules**)

- **Associated Physical Abnormalities**

- **Skeletal anomalies (absent radii, but normal thumbs):** TAR Syndrome
- **Dysmorphic features, developmental delay:** Jacobsen Syndrome (Paris-Trousseau)

- **Family History of Bleeding or Thrombocytopenia**

- **Family history of chronic thrombocytopenia** suggests an inherited disorder.

- **Failure to Respond to Platelet Transfusion**

Etiology of Inherited Thrombocytopenia

Normal size platelet

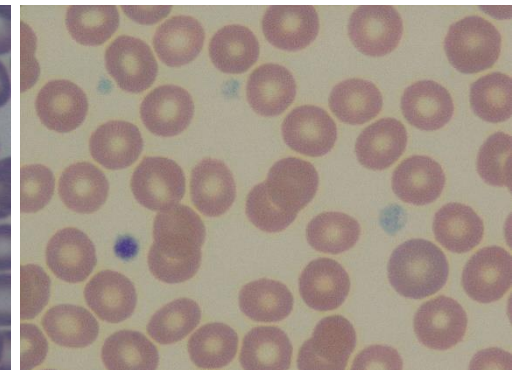
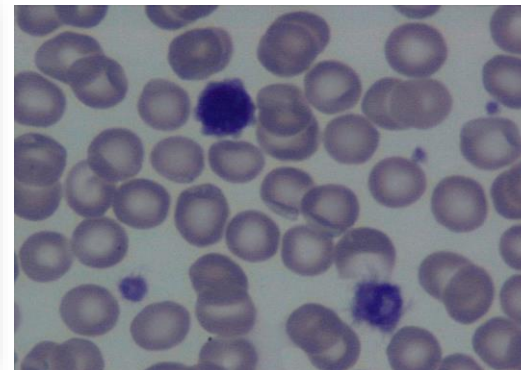
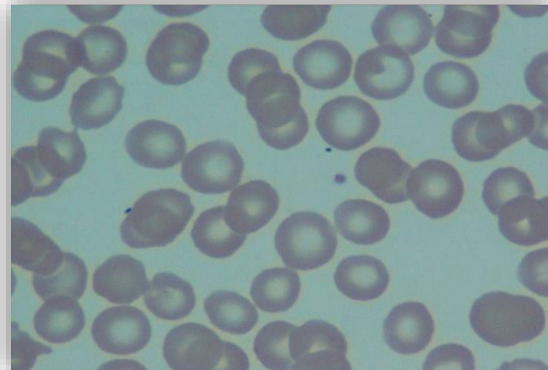
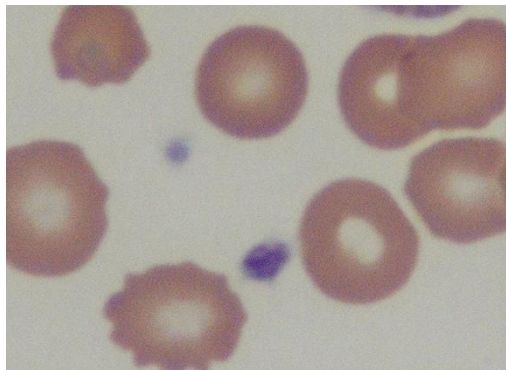
- Congenital amagakaryocytic thrombocytopenia
- Thrombocytopenia-absent radius (TAR) syndrome
- Familial platelet disorder with predisposition to myeloid malignancy

Small size

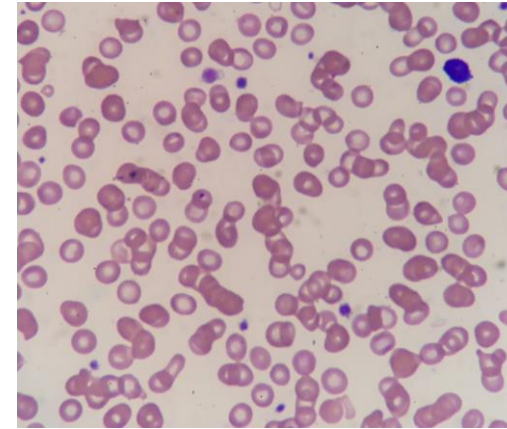
- Wiskott Aldrich syndrome
- X-linked thrombocytopenia

Large size

- MYH9
- Bernard Soulier syndrome
- Glanzmann thrombastenia
- vWD type 2B
- Digeorge syndrome
- Gray platelet syndrome
- ACTN1, TUBB1, GFI1B



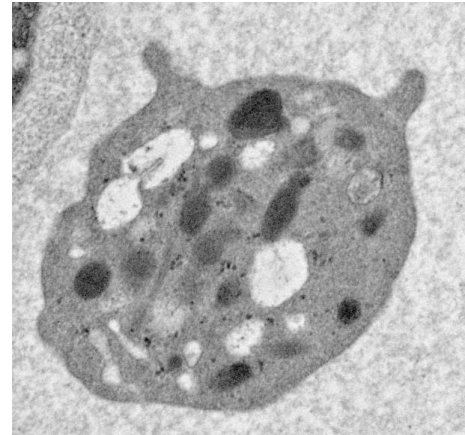
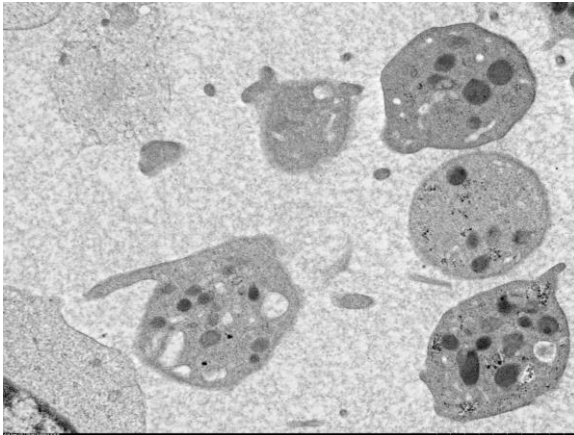
Paris-Trousseau Jacobsen Syndrome



Chromosome (11q23.3)

- Retrognathia
- Prominent forehead
- Broad nasal bridge
- V shape face

GG



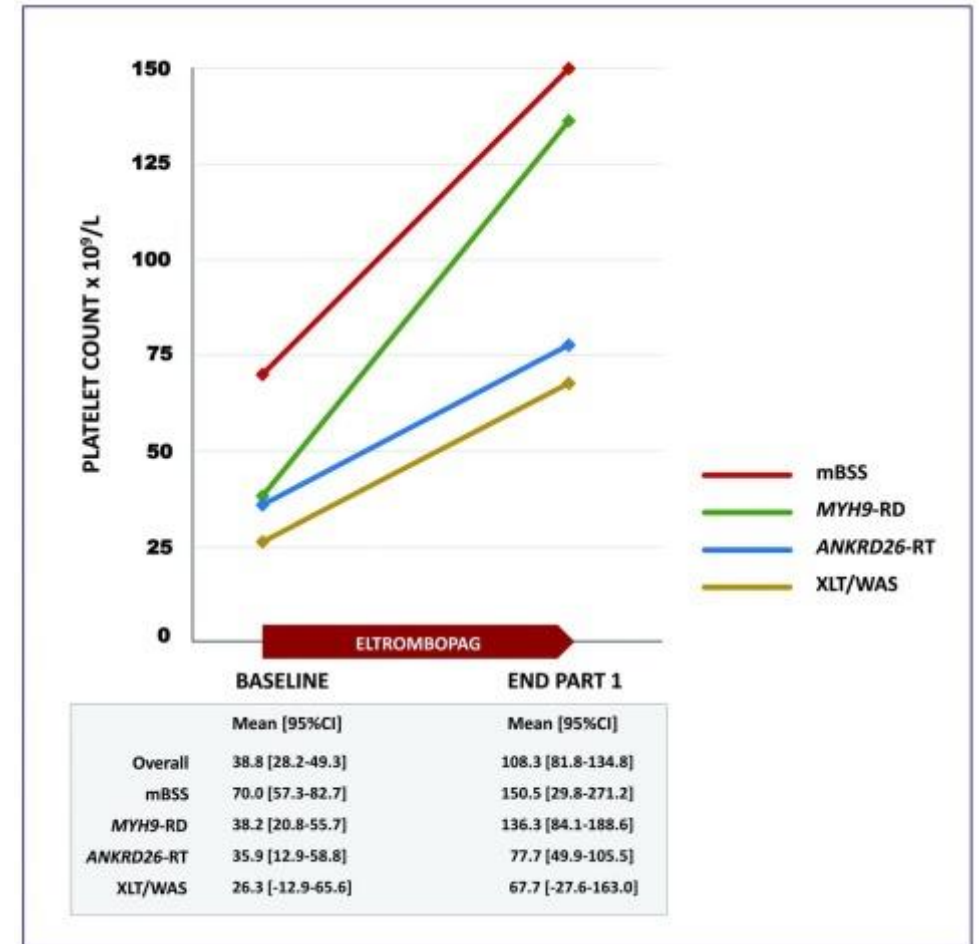
Involve gene FLI1 associate
with thrombocytopenia

Thrombopoietin Receptor Agonist in Congenital Thrombocytopenia

- TPO-RAs have shown benefit in the treatment of congenital thrombocytopenia
 - WAS
 - ANKRD26-related thrombocytopenia
 - MYH9-related disease

	Overall	MYH9-RD	ANKRD26-RT	XLT/WAS	mBSS	ITGB3-RT
Patients, n.	24	9	9	3	2	1
MF, n. of patients	14/10	2/7	7/2	3/0	2/0	0/1
Age, years - mean [SD]	41.1 [13.7]	42.9 [14.7]	40.9 [15.1]	29.3 [6.8]	50 [5.7]	45 [-]
Platelet count, ¹ x10 ⁹ /L - mean [SD]	40.1 [22.4]	38.2 [22.7]	37.4 [22.2]	26.3 [15.8]	70 [14]	62 [-]
Spontaneous bleeding, ² n. of patients	13	3	7	1	1	1
WHO grade = 1, n.	7	0	7	0	0	0
WHO grade = 2, n.	4	1	0	1	1	1
WHO grade = 3, n.	2	2	0	0	0	0
Previous splenectomy, ³ n. of patients	2	2	0	0	0	0

¹As evaluated by phase-contrast microscopy in a counting chamber. ²Spontaneous bleeding during the week preceding the baseline evaluation according to the World Health Organization bleeding scale. ³Previous splenectomy because of a mistaken diagnosis of immune thrombocytopenia. MYH9-RD: MYH9-related disease; ANKRD26-RT: ANKRD26-related thrombocytopenia; XLT/WAS: X-linked thrombocytopenia/Wiskott-Aldrich syndrome; mBSS: monoallelic Bernard-Soulier syndrome; ITGB3-RT: ITGB3-related thrombocytopenia; n: number; M: male; F: female; SD: standard deviation; WHO: World Health Organization.



Makris M. Thrombopoietin receptor agonists for the treatment of inherited thrombocytopenia. Haematologica. 2020;105:536-538.

Zaninetti C, et al. Eltrombopag for the treatment of inherited thrombocytopenias: a phase II clinical trial. Haematologica. 2020;105:820-828.

Summary

- Thrombocytopenia commonly occurs in neonate
- Immune thrombocytopenia usually occurs within 72 hours after birth
- There is a risk of serious hemorrhage in neonates with severe thrombocytopenia ($<30,000/\mu\text{L}$)
- Threshold for platelet transfusion is $<25,000/\mu\text{L}$
- Advance in the prevention of HPA-1-a alloimmune thrombocytopenia: hyperimmune anti-HPA-1a IgG, FcRn receptor blockage, and TPO-RA
- Congenital thrombocytopenia should be aware in neonates with sustained thrombocytopenia

Thank You