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MEDICINE

Fetal/Neonatal Alloimmune Thrombocytopenia (FNAIT)

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Objectives

- Epidemiology and pathophysiology of FNAIT
- Testing algorithm
- Treatment/prevention
- Compare & contrast with hemolytic disease of the fetus/newborn (HDFN)
- Time for questions & discussion

Illustrative case

- 1 day old full term male born to a healthy 33 y/o Caucasian mother (G1P0) with normal pre-natal labs
- Pediatrician in the newborn nursery noted petechiae on his chest during her initial exam
- CBC: 19.3 \22.4/ 34 000
- Consult Pediatric Hematology for diagnosis and management



Differential diagnosis of thrombocytopenia in a neonate

- Autoimmune thrombocytopenia (maternal ITP, SLE)
- Drug-induced thrombocytopenia
- Hypersplenism
- Kasabach-Merritt phenomenon
- DIC
- Thrombocytopenia Absent Radii (TAR) syndrome
- Infections (viral, bacterial, fungal)
- FNAIT
- Fanconi anemia
- Congenital Amegakaryocytic Thrombocytopenia (CAMT)
- Trisomies 21, 18, 13, Turner syndrome
- Wiscott-Aldrich syndrome
- MYH-9 disorders (May-Hegglin)
- Bernard-Soulier syndrome
- Pre-eclampsia, birth asphyxia
- Infiltrative disorder of bone marrow (neonatal leukemia, neuroblastoma)



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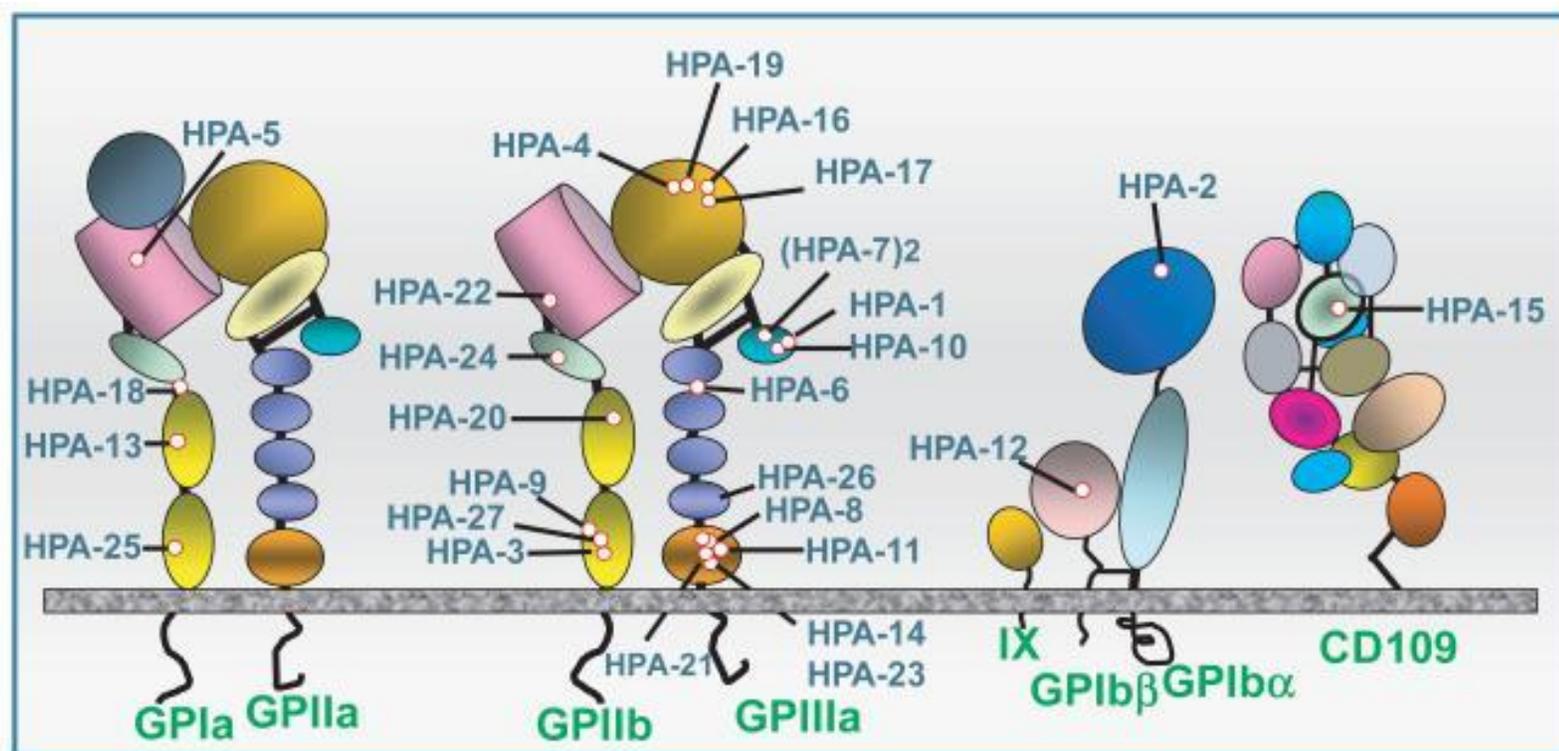
- FNAIT = leading cause of severe thrombocytopenia in the fetus & neonate
- Leading cause of intracranial hemorrhage in the full term infant
 - Incidence estimated between 7 – 25%
 - Most occur before 28 weeks in utero
- Several large prospective studies of women negative for HPA-1a showed that between 1/1000 and 2/1000 HPA-1a positive infants had FNAIT caused by maternal HPA-1a antibodies
- Common for immunization against platelet alloantigens to occur *during first pregnancy* – common for a firstborn infant to be affected by FNAIT

- Severely affected infant will present with severe petechiae & purpura, severe thrombocytopenia
- No other explanation found for thrombocytopenia (especially infectious causes)
- Infant born to a mother who previously gave birth to an infant with FNAIT tends to have more severe disease than its older sibling

Intracranial hemorrhage (ICH)

- Occurs in 10-20% of symptomatic infants
- Up to 80% of ICH occur prenatally
- After delivery, greatest risk for ICH in first 96 hours of life

Platelet specific antigens



← Platelet membrane glycoproteins (GPs)

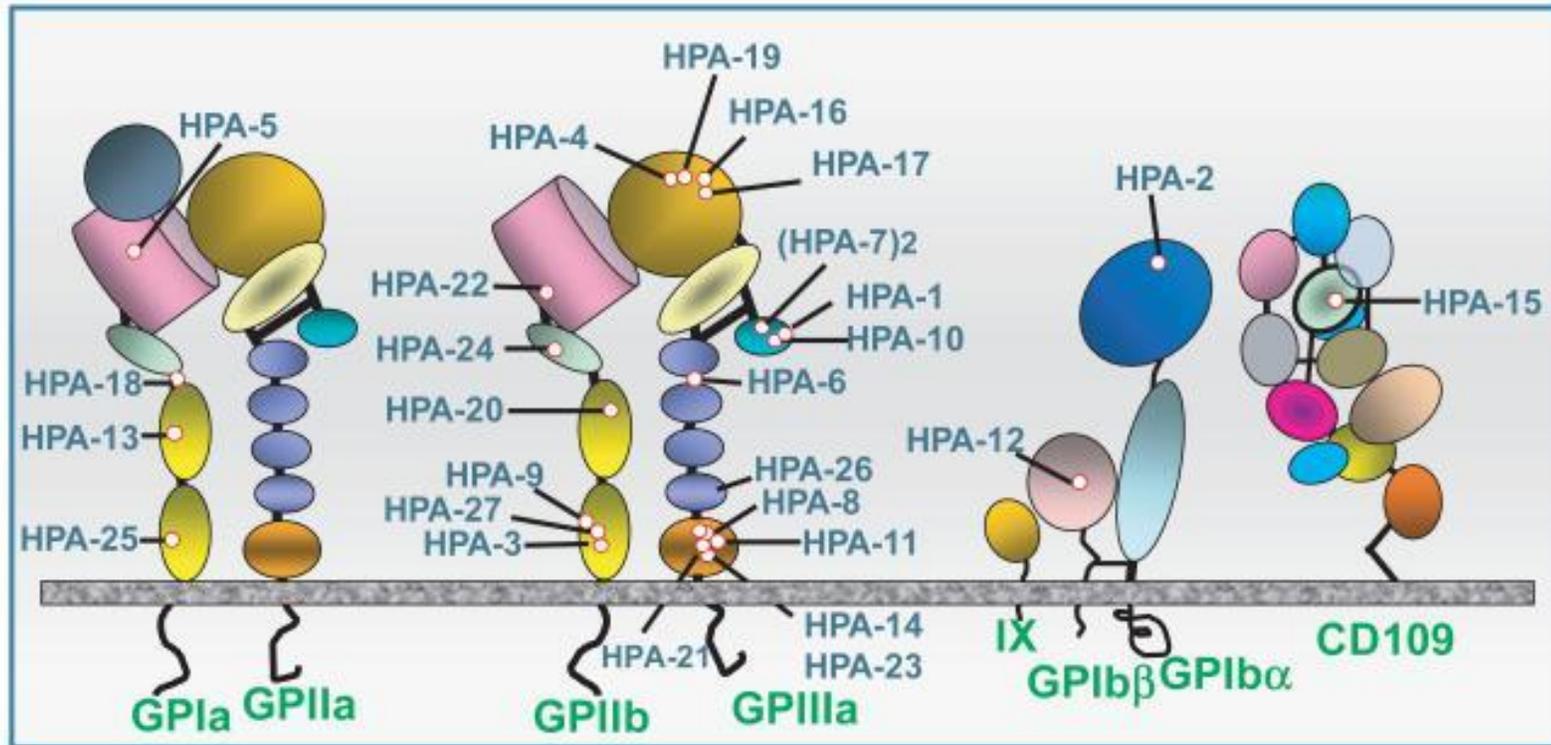
Collagen receptor

Fibrinogen receptor

Von Willebrand
receptor

Uncertain
function

Platelet specific antigens



Genetic polymorphisms resulting from *at least 27* single amino acid substitutions located on 6 different glycoproteins have been shown to cause FNAIT

Human Platelet Antigen (HPA)

- Nomenclature developed by international consensus
- Antigen systems designated HPA-1, HPA-2, etc. more or less in order of their discovery
- More common allele = 'a'
- Less common allele = 'b'
- HPA database – <http://www.ebi.ac.uk/ipd/hpa/index.html>

HPA-1 antigen system

- First HPA implicated in FNAIT (Shulman, 1962)
- Maternal-fetal incompatibility for HPA-1a by far the most common cause of NAIT in families of Caucasian & African ancestry
 - Account for 85% of cases in which HPA-specific antibody is identified
- Only 2% of women are HPA-1a negative (i.e. HPA-1b/b)

Other antigens implicated in FNAIT

- 95% of serologically confirmed cases of FNAIT in Caucasian families are caused by maternal immunization against antigens belonging to:
 - HPA-1
 - HPA-2
 - HPA-3
 - HPA-5
 - HPA-15
- 23 less common mutations identified in cases of apparent FNAIT where antibodies specific for these 'common' HPA antigens are not detected
 - Some described in single case reports



ABO antigens also rarely implicated in FNAIT

- Platelets express small quantities of A and B antigens on their surface
- 5% of people possess platelets that have *unusually* large numbers of A or B antigens
- Some infants with high expression are at risk of thrombocytopenia from maternal ABO incompatibility (same infants at risk of ABO hemolytic disease of the newborn)

Other rarer causes of FNAIT

- CD36 expressed on platelets, RBCs, endothelial cells, other tissues
 - 5% of people with Asian or African ancestry do not express CD36
 - Maternal immunization against CD36 reported
- Platelets express 20 000 copies of HLA class I antigens
 - 30% of multiparous women have HLA class I antibodies
 - Anti-HLA class I antibody mediated FNAIT has been reported (though not nearly as often as expected given frequency of women with these antibodies)

- Serologic investigation is absolutely required, even in infants only mildly affected, because results are *critical* for management of mother's subsequent pregnancies
- Proper lab diagnosis is sophisticated, requires a thorough understanding of platelet serology, and often a communication between lab director and attending physician
- Need blood samples from both *mother* and *biologic father* (not the infant)

Platelet glycoproteins

- DNA typing
- Variety of methods for platelet genotyping:
 - Allele-specific PCR (primers attached to single nucleotide polymorphisms)
 - Melting curve analysis
 - 5'-nuclease or *Taqman* assay
 - High-throughput platelet genotyping methods

Platelet reactive antibodies

- Combination of methods to ensure a thorough work up since *no single method is sufficient*
- Assay that uses intact platelets to screen serum or plasma for antibodies
- GP antigen capture assay to identify the antigen that the antibodies target

Platelet reactive antibodies, continued

- Flow cytometry used to detect antibodies
 - Secondary probes for IgG and IgM used to test maternal serum against washed paternal and maternal platelets and a small panel of platelets from normal group O donors for selected common HPA antigens
- Solid phase assays to identify glycoprotein for which maternal antibody is specific
 - Modified antigen capture enzyme-linked immunosorbent assay (MACE ELISA)
 - Monoclonal antibody immobilization of platelet antigens (MAIPA) widely used in Europe

Interpretation of test results

- In 20 – 35% of cases, an antibody specific for HPA antigen present in the father but not the mother is found in maternal serum
 - HPA-1a is targeted in 75-90%
 - HPA-5b in 8-15%
 - HPA-1b in 1-4%
 - HPA-3a in 1-2%
 - HPA-5a in 1%
- Occasionally, two HPA antibodies found

Unresolved cases

- 2/3 suspected FNAIT cases sent to Blood Center of Wisconsin are *not resolved* based on maternal-fetal incompatibility
- In some, thrombocytopenia secondary to a non-immune cause
- Low avidity HPA antibodies active area of research

Our case results

- Platelet antigen typing of the mother:
 - HPA 1b/1b HPA 2a/2a HPA 3a/3a HPA 4a/4a HPA 5a/5a HPA 6a/6a HPA 9a/9a HPA 15a/15a
- Platelet antigen typing of the father:
 - HPA 1a/1a HPA 2a/2a HPA 3a/3a HPA 4a/a HPA 5a/5a HPA 6a/6a HPA 9a/9a HPA 15a/15a

Results, continued

- Platelet antibody screen:

Platelet target	IgG result	IgM result
Target platelet 1	+	-
Target platelet 2	-	-
Mother's platelets	-	-
Father's platelets	+	-

Results, continued

- Platelet antibody identification:

Class I HLA	Pool 1b/IX	Pool IV	HPA 1a/1a – 3a/3a	HPA 1b/1b – 3b/3b	HPA 5a/5a
-	-	-	+	-	-

Results, continued

- Modified antigen capture ELISA crossmatch:

Father's platelets (IIb/IIIa – GP1 locus)

+

- ‘Strong positive reactions detected in mother’s serum against HPA-1a positive platelets only. The platelet typing studies, together with the serologic results support a diagnosis of NAIT due to the incompatibility for HPA-1a in this family. Since the father is homozygous for HPA-1a, subsequent pregnancies in this family are at extremely high risk (approaching 100%) of being affected with NAIT. In the event of future pregnancies are contemplated, genetic counseling would be appropriate.’

- Random donor platelet transfusion ASAP
 - ABO compatible
 - CMV negative
 - Irradiated
- Will elevate platelet count transiently *even if* incompatible with maternal antibody
- Some blood banks have HPA-1b/b platelets available (time for collection?)
- IVIG lessens overall period of thrombocytopenia and prolongs survival of incompatible platelets

- HPA-compatible platelets can be collected from the mother and washed in unusual cases requiring transfusion support over an extended period of time
- Must be irradiated
- MUST BE WASHED to remove antibody-containing maternal plasma (multiple reports where this step is missed and neonatal thrombocytopenia lasts for weeks to months)

Management of subsequent pregnancies

- Is the infant incompatible with maternal alloantibody previously demonstrated?
- What is the risk of antenatal ICH (what is the fetal platelet count)?
- Offer risk-stratified antenatal therapy to the mother

Is the infant incompatible with maternal alloantibody previously demonstrated?

- HPA genotype of father
 - Homozygous → infant IS incompatible
 - Heterozygous → infant has 50% of being incompatible
 - Fetal genotyping (CVS or amniocentesis)

What is the risk of antenatal ICH (what is the fetal platelet count)?

- Non-invasive methods:
 - Testing of mother's serum for strength of anti-HPA antibody
 - Severity of FNAIT in previously affected sibling(s)
- Invasive fetal blood sampling

Risk-stratified antenatal therapy

Stratum 1 – history of previous fetus or newborn with thrombocytopenia or ICH of unknown etiology, no HPA antibody detected	Monitor with serial maternal testing to detect HPA antibodies (including serological crossmatching with paternal platelets) at 12, 24, 30 weeks
Stratum 2 – history of previous fetus or newborn with serologically confirmed FNAIT having only thrombocytopenia and no evidence of ICH	IVIg and steroid therapy starting at 20 weeks gestation; elective C/S at 37 – 38 weeks
Stratum 3 – history of serologically confirmed FNAIT and previous fetus or newborn with ICH at 28 weeks of gestation or more	IVIg and steroid therapy starting at 12 weeks; elective C/S at 37 – 38 weeks
Stratum 4 – history of serologically confirmed FNAIT and previous fetus or newborn with ICH < 28 weeks	High dose IVIg and steroid therapy starting at 12 weeks; elective C/S at 37 – 38 weeks

Screening?

- Pregnant women could be screened for HPA type and tested for alloantibodies
- High disease burden for affected child and family
- High costs for health care & society in case of ICH
- Prevention of even a few cases could be cost effective

Novel non-invasive screening

- Non-invasive prenatal diagnostics (NIPD) used to screen for: single gene disorders, chromosomal disorders (trisomies), fetal sex
 - Largely replaced invasive testing via CVS or amniocentesis
- Fetal DNA can be obtained from maternal plasma and tested for HPA-1a (majority of fetuses will have at least one HPA-1a allele)
- Anti-HPA-1a (B2G1 Deltanab) being considered for clinical trials in Norway
 - Eliminate HPA-1a positive platelets from maternal circulation analogous to Rhogam

Compare/contrast with HDN

Characteristic	FNAIT	HDN
Parity of mother	<i>Can occur in first pregnancy</i>	Must have been sensitized to RBC antigen (prior pregnancy)
Antibody	IgG to <u>HPA antigens</u> , others	IgG to RBC antigens (anti-D, anti-c, anti-K most severe)
Clinical presentation	Thrombocytopenia; ICH usually < 28 weeks	Severe intrauterine anemia; hydrops fetalis (demise)
Treatment	Random donor platelets and IVIG	Intrauterine pRBCs
Prevention	Antenatal risk & appropriate management; anti-HPA 1a?	Rhogam; K neg pRBCs for all women?

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Questions/Comments

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