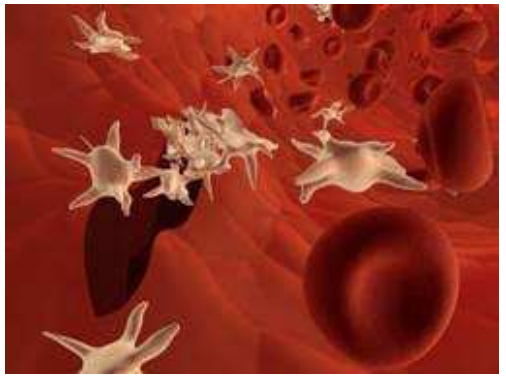
Gina Folk, MLS(ASCP)CMSBBCM

Community Blood Center

Family Ties

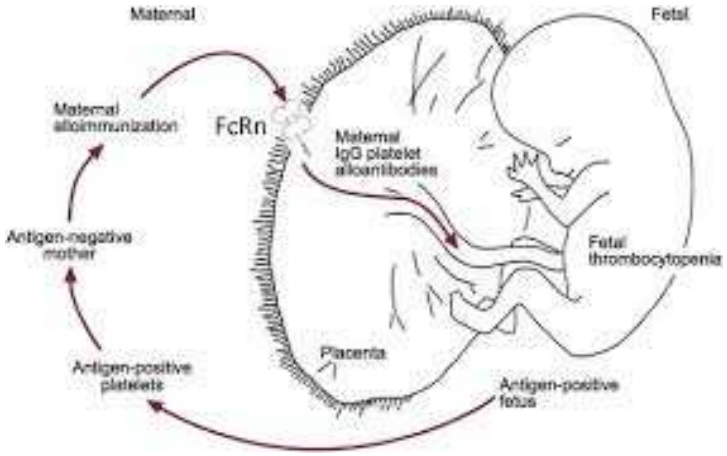
A Platelet Story

Thrombocytopenia



- Definition: Platelet count of <150 X 10^9/L
- Most frequent hematologic disorder in neonates admitted to NICU
  - 18-35% incidence
- Classification
  - Mild (100-149 X 10^9/L)
  - Moderate (50-99 X 10^9/L)
  - Severe (<50 x 10^9/L)
- Data may not be as accurate as testing usually performed on premature newborns

Fetal and Neonatal Alloimmune Thrombocytopenia (FNAIT)



- Mom is exposed to incompatible, paternally derived human platelet antigen (HPA) or HLA antigens on fetal platelets.

- HPA-alloantibodies enter fetal circulation after crossing the placenta
  - Destruction of fetal platelets
  - Damage to endothelial cells ⟹ can lead to bleeding complications

- Bleeding ranges from minor skin manifestations to severe intracranial hemorrhages (ICH) or even death
  - Severe hemorrhages are rare (1 in 11,000 newborns)

Diagnosis

- Often diagnosed by symptoms
- Bleeding complications after birth most common symptom
- Occasionally, identified due to the mother's sister or another family member with a pregnancy complicated by FNAIT
- Diagnostic workup should be completed to help with serologic diagnosis
- Serological FNAIT diagnosis confirmed in cases of:
  - Maternal-neonatal incompatibility
  - Maternal-paternal incompatibility

Determination of FNAIT Risk

- Not every pregnancy will have FNAIT
  - First step is to determine whether incompatibility is present

  - Paternal genotype will greatly determine risk
    - If father is homozygous for prior HPA or HLA antigen then every pregnancy is at risk

    - If father is heterozygous for prior HPA or HLA antigen then there is a 50% chance of passing on the allele positive for the antigen
      - Testing of cell free fetal DNA in maternal plasma
      - Amniocentesis can also be performed to obtain fetal DNA

Antenatal Treatment

- Goal: to prevent bleeding complications
- Not focusing on platelet count or preventing thrombocytopenia
  - Even extremely low platelet counts do not necessarily result in clinical bleeding
- Low platelet count ≠ severe bleeding
  - Often, other pathological mechanisms that play a role in bleeding problems
- Platelet counts should not be the only thing being monitored and treated during a FNAIT pregnancy
- Different treatment options include:
  - Platelet transfusion
  - Intravenous immunoglobulins (IVIG)
  - Corticosteroids

Postnatal Management

- Goal:
  - Prevent or stop neonatal thrombocytopenic hemorrhage
- Lab Evaluation:
  - Cord blood tested to assess severity of thrombocytopenia
- Results will help determine best course of treatment
  - Platelet transfusion
  - IVIG
  - Other options
    - Corticosteroids
    - Exchange transfusions

What to Transfuse

- If confirmed NAIT diagnosis:
  - Ideally, platelets negative for the responsible platelet alloantibody
  - 90% of cases are due to HPA-1a
    - HPA-1a negative platelets

- When platelet alloantibody is undetermined or units are unavailable:
  - Random platelets with/without washing the platelets
  - Never withhold transfusion waiting for antigen negative platelets

- Maternal platelets
  - Often need to be washed and irradiated, potentially leading to destroyed platelets from manipulation and it is time consuming

- No agreement on the “safest” platelet to transfuse

IVIG

- For Use when
  - Mechanism is unknown
    - Theory: Peripheral platelet destruction is inhibited through changes in Fc receptors on platelets and macrophages

- Dose: 1-2 g/kg for 2-5 consecutive days

- Retrospective cohort analysis has shown that IVIG treatment has a 65% response rate

- Disadvantage: takes longer to work than platelet transfusion
  - Effects are not seen until at least 24-48 hours after IVIG infusion

- IVIG not recommended as first line therapy alone in NAIT cases
  - Often given when random platelet transfusion is only option available in combination with IVIG
    - Helps prolong the effect of transfusion

Other Options

- Corticosteroids
  - Dose of 1 mg methylprednisolone, intravenous every 8 hours
  - Unsure if use is beneficial
    - Lack of evidence and possible side effects

- Exchange Transfusions
  - Removal of harmful maternal circulating antibodies from fetal circulation
  - Usually used only in very urgent situations
    - No platelets available and treatment with IVIG effects takes too long
  - Therapy is no longer used
    - High risk of (bleeding) complications, especially in thrombocytopenic neonates

CBC Patient

- 6/15/2018 Hospital calls to say sending a NAIT workup
- Mom has a history at CBC
  - Idiopathic Thrombocytopenic Purpura (ITP) with splenectomy
  - 4/30/2012-Positive platelet antibody screen
    - Reactive with 6 of 8 (75%) of a panel of fresh random donor platelets by solid phase red cell adherence assay (SPRCA)
    - HLA class I antibody was detected by solid phase ELISA
    - Sent 10 crossmatch platelets for baby between 5/3/2012-5/29/2012
  - 5/30/2012- Platelet antibody screen repeated—Negative
    - Retested by hospital request
    - Testing performed only by solid phase ELISA assay

Testing on Current Sample (NAIT Workup)

- ABO
  - Mom = A
  - Dad = O+

- Antibody Screen
  - Current platelet antibody screen negative
    - Tested using a panel of fresh random donor platelets by SPRCA and tested using a panel of 13 dried phenotyped platelets by SPRCA.

- Platelet Bound IgG (PBIgG)
  - Mom = Negative
  - Dad = Negative

- Crossmatch (Maternal vs Paternal)
  - Mom's plasma against dad's platelets
  - Positive

Further Testing

- Baby Boy was still not getting bump from transfusion of random platelets so hospital requested crossmatch platelets for baby
  - Sent 1 crossmatch platelet on 6/20/2018

- Requested antibody screen be performed on baby
  - Antibody screen was negative by both methods of SPRCA testing
    - Additional crossmatched platelet sent 6/22/2018

- HLA and HPA testing requested on mom, dad and baby.

New York *Blood Center*

Laboratory of Immunohematology and Genomics
45-01 Vernon Blvd., Long Island City, N.Y. 11101
718-752-4771 • Fax 718-752-4747

Date Reported: 07/11/18

Community Blood Center – Kansas City
Reference Laboratory
4040 Main Street
Kansas City, MO 64111

SAMPLES: Date received: 07/09/18 Sample date: 07/05/18

[redacted]

HISTORY: Hispanic family; NIT. Maternal sample has negative platelet antibody screen but is incompatible with paternal platelets.

TESTING REQUESTED: Genotype for HPA-1 (*HPA-1*), HPA-3 (*HPA-3*) and HPA-5 (*HPA-5*).

TESTING PERFORMED: HPA 2.1 BeadChip Genotype which includes HPA-1 through HPA-9, HPA-11 and HPA-15.

HPA RESULTS:

	HPA RESULTS																				
Sample	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a	7b	8a	8b	9a	9b	11a	11b	15a	15b
Maternal	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0
Paternal	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+
Infant	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0

+ = positive
0 = negative

COMMENTS: Based on HPA genotyping, the paternal sample is positive for HPA-15b while the maternal sample is negative. The mother and infant have the same HPA profile.

Sunitha Vege, MS
Manager, Genomics

Connie Westhoff

Connie M Westhoff, MT(ASCP)SBB, PhD
Exec. Scientific Director Immunohematology Genomics

These *in vitro* diagnostic tests were developed and their performance characteristics established in the Genomics Laboratory. The tests have not been submitted to the Food and Drug Administration (FDA) for clearance or approval and; therefore, are not FDA-licensed tests. The Blood Group Genomics Laboratory is certified under the Clinical Laboratory Improvement Amendment (CLIA) of 1988 as qualified to perform high complexity clinical testing. The New York Blood Center has been approved by the New York State Department of Health to perform these tests under its current Clinical Laboratory Permit.

These results are intended to predict a blood group antigen profile in a patient or donor, and are not intended for clinical diagnosis or as the sole means for patient management decisions. There are situations where testing DNA of a person may not reflect the red cell phenotype and not all performance characteristics have been determined. Nucleotide changes that inactivate gene expression or rare new variant alleles may not be identified in these assays. In addition, test results obtained from DNA isolated from leucocytes and other hematopoietic cells may differ from DNA isolated from other tissues in persons with a history of transplantation.

Laboratory Director – Dr. Connie Westhoff, MT(ASCP)SBB, PhD

MA2037-18, 2038-18, and 2039-18

Maternal HLA Results

Locus	ALLELE 1	Rare Alleles	NMDP Code	ALLELE 2	Rare Alleles	NMDP Code
A*	02:BCZGU		BCZGU	29:02:03		
B*	35:BDDGD		BDDGD	45:HVBN		HVBN
C*	06:BCSZB		BCSZB	07:BFKCD		BFKCD
DRB1*	04:HTWY		HTWY	12:AYTDJ		AYTDJ

Paternal HLA Results

Locus	ALLELE 1	Rare Alleles	NMDP Code	ALLELE 2	Rare Alleles	NMDP Code
A*	24:AYRZE		AYRZE	24:AYRZE		AYRZE
B*	39:06:02			39:06:02		
C*	07:BCZHT		BCZHT	07:BCZHT		BCZHT
DRB1*	14:06:01			14:06:01		

Infant HLA Results

Locus	ALLELE 1	Rare Alleles	NMDP Code	ALLELE 2	Rare Alleles	NMDP Code
A*	24:02:01G		AYRZE	29:02:03		
B*	39:06:02			45:01:01G		DCFY
C*	06:02:01G	06:04:01 06:04:02 06:11 06:31 06:47 06:122 06:136	BCSZB	07:02:01G	07:138 07:37 07:76:01 07:241 07:27:01 07:49 07:31:01	BFKCD
DRB1*	12:01:01G	12:03:03 12:09	BGAKJ	14:06:01	14:29 14:177	BGAKK

Results

- HPA Genotyping
  - Only difference, paternal sample is positive for HPA-15b
  - Infant is negative for HPA-15b
  - Maternal and infant sample have same HPA profile

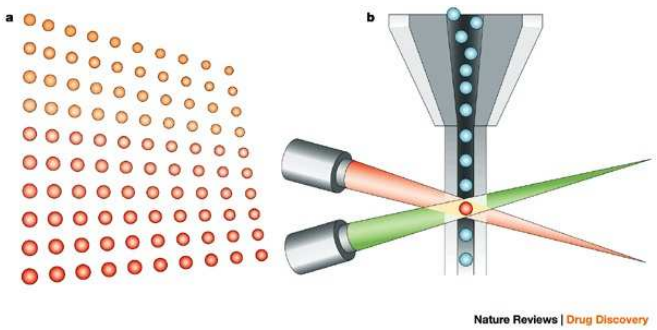
- HLA

	A*		B*		C*		DRB1*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Maternal	02:BCZGU	29:02:03	35:BDDGD	45:HVBN	06:BCSZB	07:BFKCD	04:HTWY	12:AYTDJ
Paternal	24:AYRZE	24:AYRZE	39:06:02	39:06:02	07:BCZHT	07:BCZHT	14:06:01	14:06:01
Infant	24:02:01G (AYRZE)	29:02:03	39:06:02	45:01:01G	06:02:01G (BCSZB)	07:02:01G(BFKCD)	12:01:01G (BGAKJ)	14:06:01

Additional Testing

- As of 7/4/2018
  - Baby still is not getting a good bump even after receiving 5 platelets
    - 2 crossmatch compatible
    - 3 random donor

- Sending maternal sample off to Rhode Island Blood Center
  - HLA antibody testing
    - Luminex-based assay: detects and identifies IgG antibodies to HLA class I antigens



Rhode Island Blood Center
405 Promenade St.
Providence, RI 02908

CLIA #: 41D0670559
ASHI #:01-1-RI-01-1

Rhode Island Blood Center

HISTOCOMPATIBILITY REPORT

To: [redacted]

Name: [redacted]
RIBC ID: 30954
DOB: [redacted]
DIAGNOSIS: N/A
MR#: N/A

Report Date: July 18, 2018

Name & DOB	Source Collected	ABO	Relation	A	B	Bw	C	DRB1*	DRB3/4/5 DR51/52/53	DQB1*
[redacted]	Unknown	Unknown	Related Donor	*	*	NT	*	*	NT	*
				*	*	NT	*	*	NT	*

If applicable, serological equivalents indicated in parenthesis. HLA-A, -B, -C, -DRB1 and -DQB1 were typed using PCR-SSO and/or PCR-SSP techniques. When ambiguous typing combinations exist, Common Well Documented (CWD) alleles are reported with the most frequent allele listed first. Individual ambiguous allele combinations will be provided upon request. NT = Not Tested.

HLA ANTIBODY ANALYSIS

Sample# / Date	Titer	DSA	Class I Specificities	Class II Specificities	cPRA	Comment
Sample #: 52459 Collected: 07/16/2018 Received: 07/17/2018 Tested: 07/17/2018 & 07/18/2018 (treated)	Neat		B7,8,18,27,37,38,39,41,42, 54,55,59,60,61,64,67,81 Cannot rule out Allele specific A*30:04;B*44:10,B*82:01 C*03:02,C*12:03 &C*15:05	None	68%	

HLA Antibody detection is performed by Luminex-based IgG antigen bead assay. Donor Specific Antibodies (DSAs) are indicated in **bold**. For each **DSA** detected, mean MFI may be requested. RIBC considers MFI values > 1000MFI to be clinically significant; however, MFI values <1000 MFI may be significant based on clinical setting.

INTERPRETATION/COMMENTS:

[redacted] serum sample collected 07/16/2018 is positive for HLA Class I antibodies.
*High resolution HLA typing was performed by New York Blood Center. Please see attached report.

Carolyn T. Young, M.D
Carolyn Te Young, MD
Chief Medical Officer

All tests were validated by and their performance characteristics confirmed by the Rhode Island Blood Center Histocompatibility Laboratory. HLA Typing and Solid Phase Multiantigen Antibody Identification have been cleared by the US Food and Drug Administration. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-1988) as qualified to perform high complexity clinical laboratory testing. Laboratory Director: Carolyn Te Young, MD

HLA Antibody Results

- Maternal sample is positive for HLA class I antibodies.
  - Has a cPRA of 68%
- Mom has antibodies to
  - B7, 8, 18, 27, 37, 38, 39, 41, 42, 54, 55, 59, 60, 61, 67, 81

	A*		B*		C*		DRB1*	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Maternal	02:BCZGU	29:02:03	35:BDDGD	45:HVBN	06:BCSZB	07:BFKCD	04:HTWY	12:AYTDJ
Paternal	24:AYRZE	24:AYRZE	39:06:02	39:06:02	07:BCZHT	07:BCZHT	14:06:01	14:06:01
Infant	24:02:01G (AYRZE)	29:02:03	39:06:02	45:01:01G	06:02:01G (BCSZB)	07:02:01G (BFKCD)	12:01:01G (BGAKJ)	14:06:01

What About Baby?

- On 7/25/18 platelet count appeared to be holding steady
- Last platelet transfusion was on 7/11/2018
  - Platelet counts:
    - 7/17/18--- 40,000
    - 7/18/2018--- 37,000
    - 7/20/18--- 49,000

- Classification
  - Mild (100-149 X 10^9/L)
  - Moderate (50-99 X 10^9/L)
  - Severe (<50 x 10^9/L)

- Vitamin and iron supplementation were given to try and help baby
- Baby was also given IVIG and corticosteroids
- Baby was discharged on 7/20/2018
- Recent follow up visit:
  - Baby was doing well and had a platelet count of 100,000

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Persistent neonatal thrombocytopenia can be caused by IgA antiplatelet antibodies in breast milk of immune thrombocytopenic mothers

Hagit Hauschner, Nurit Rosenberg, Uri Seligsohn, Rafael Mendelsohn, Aryeh Simmonds, Yakov Shiff, Yaakov Schachter, Shraga Aviner and Nechama Sharon

Discussion

- Evidence of antiplatelet antibodies in breast milk of ITP patients, potentially, associated with neonatal persistent thrombocytopenia.
  - Infant's immune system is immature during the first few months after birth
- IgA antibodies can be absorbed along the infant's gastrointestinal tract and enters the circulation
- Primary mechanism for platelet destruction in ITP is thought to be autoantibody-dependent phagocytosis, especially in cases of anti- $\alpha_{IIb}\beta_3$ antibodies
- Other studies
  - Kelemen et al. (1978) suggested that colostrum from ITP patients contributed to the lowering of circulating platelets counts in the first postnatal days
  - Meschengieser and Lazzari (1986) described an ITP patient who gave birth to a thrombocytopenic preterm infant who was breastfed from day 5 without apparent adverse effects on his platelet count.

Conclusion

- CBC patient:
  - Baby was getting a mixture of formula and breast milk
  - No clear evidence if IgA antibodies played a role in persistent thrombocytopenia
    - Mother's history does lead to possibility of IgA antibodies as very similar to index case in study.

- Treatment
  - Antenatal: IVIG
  - Postnatal: platelet transfusion + IVIG

- Overall conclusion
  - No firm evidence to discourage breastfeeding
  - Discontinuation of breastfeeding does present a viable solution in cases of persistent thrombocytopenia in neonate
  - Additional research is needed


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