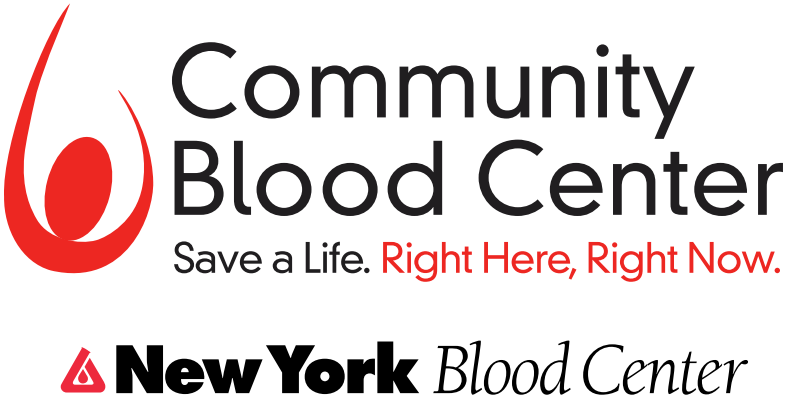
# TEST ME BABY, ONE MORE TIME



# Objectives

- Describe the testing involved when Neonatal alloimmune thrombocytopenia (NAIT) is suspected.
- Describe typical results of testing in NAIT cases.
- Explain how clinical presentation of NAIT may not correlate with laboratory test results.

# Neonatal Alloimmune Thrombocytopenia

- Involves the immune destruction of fetal platelets by maternal antibody

- A mother may become sensitized from an incompatible platelet antigen inherited from the father
- Neonates are at risk for major bleeding complication (intracranial hemorrhage)

# Neonatal Alloimmune Thrombocytopenia

- Human Platelet Alloantigens (HPA)
  - HPA antigens 1 – 11, and 15
    - "a" designated for the high frequency antigens
    - "b" designated for the low frequency antigens
- HPA's are expressed on Glycoproteins (GP's)
  - GP: Ia,IIa, IIb, IIIa, Ibα,β : More on that later
- Most causes of NAIT are due to mismatch of HPA-1a ($PL^{A1}$)
  - 98% of individuals are HPA-1a positive : More on that later

# Testing for NAIT

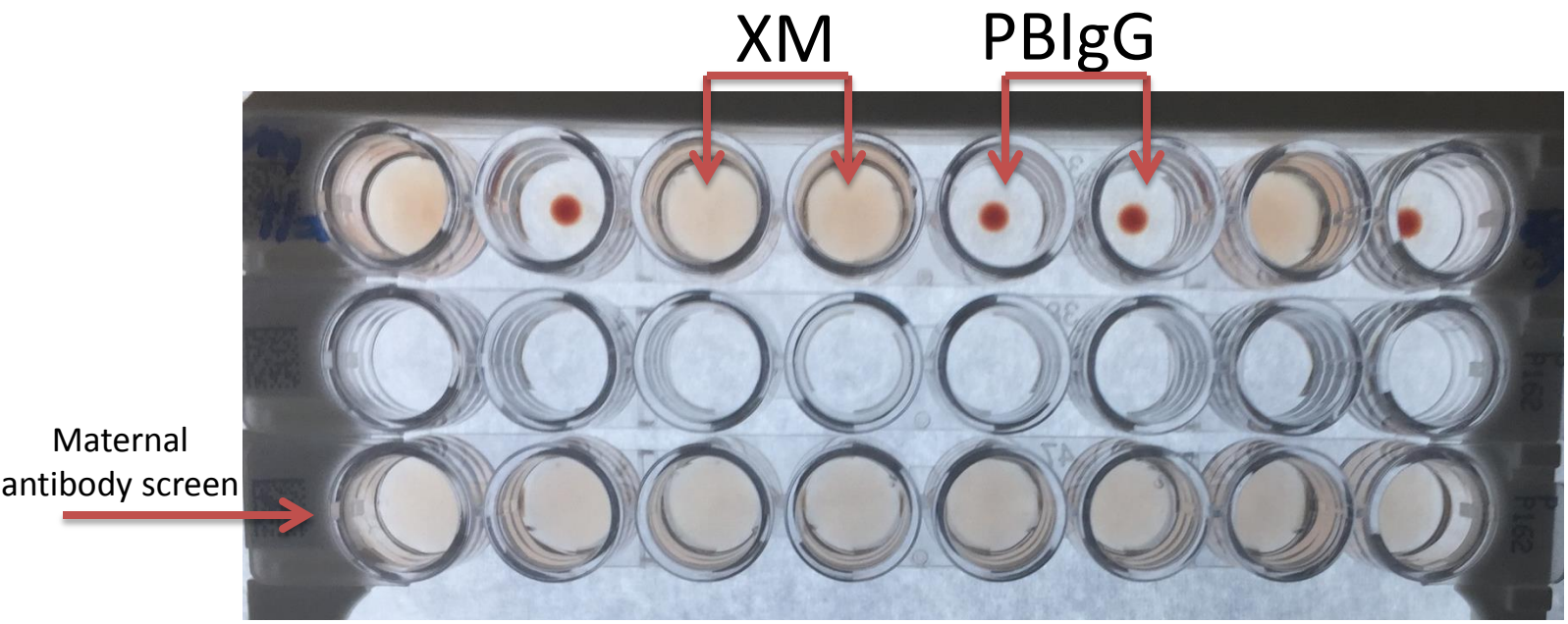
- Maternal and paternal samples needed
  - No sample needed on baby

Maternal sample testing:

- Antibody Screen
- Platelet Bound IgG – paternal testing as well
- Crossmatch with paternal platelets – neutralize if ABO incompatible
- HPA-1a typing – paternal testing as well

# Typical Results of NAIT

- Maternal antibody screen will be positive
  - Solid phase testing may show reactivity with all wells tested – tested against panel of random group O donors

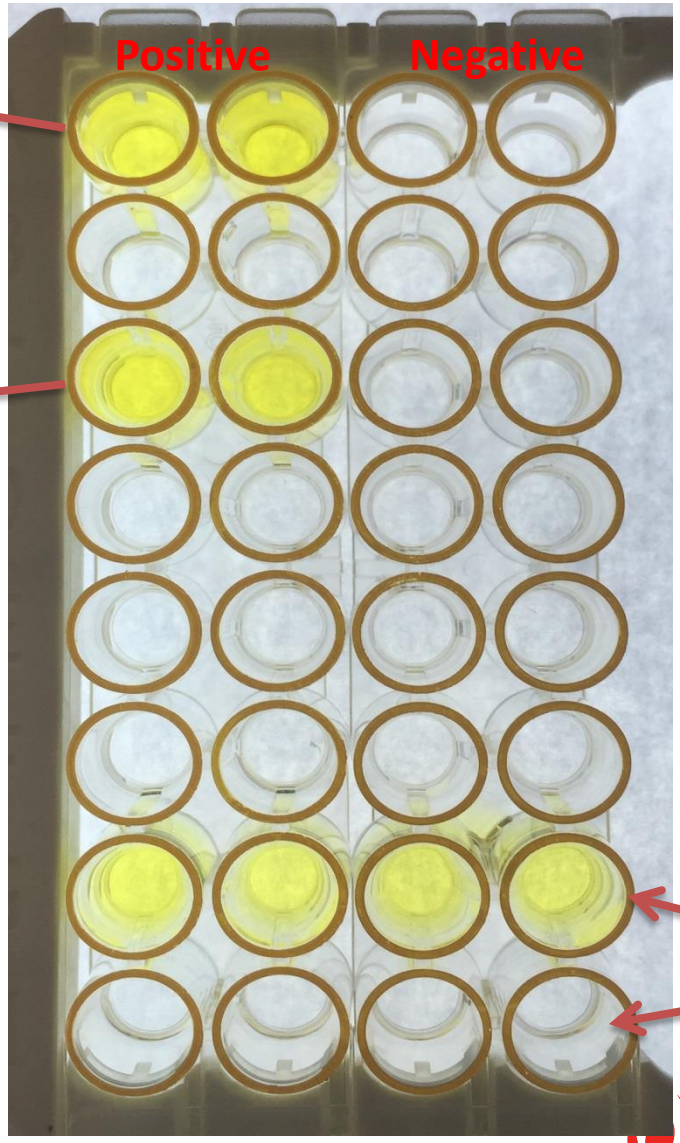


# Typical Results of NAIT

- ELISA testing will help differentiate the antibody based on the reactivity seen with different glycoproteins
  - Examples of different antigens on different glycoproteins are tested
  - Patterns will help differentiate specific antibodies to specific HPA's based on the reactivity seen
- All wells may be positive in solid phase testing, but then singled out via ELISA

# Typical Results of NAIT

| |
|---|
| GP IIb/IIIa |
| HPA-1a/1a (PIA1) |
| HPA-3a/3a (Baka) |
| HPA-4a/ (Pena) |
| |
| GP IIb/IIIa |
| HPA-1b/1b (PIA2) |
| HPA-3b/3b (Bakb) |
| HPA-4a/ (Pena) |
| |
| GP IIb/IIIa |
| HPA-1a/1a (PIA1) |
| HPA-3b/3b (Bakb) |
| HPA-4a/ (Pena) |
| |
| GP Ia/IIa |
| HPA-5b/5b (Bra) |
| |
| GP Ia/IIa |
| HPA-5a/5a (Brb) |
| |
| GP Ib/IX |
| HPA-2a/2b (Koab) |
| |
| HLA |
| CLASS I |




Pos and Neg controls

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# Typical Results of NAIT

- Another solid phase test can be performed if ELISA testing is unavailable
  - Instead of random platelets, phenotyped platelets are used to help determine the antibody present

| Well |
| --- |
| Donor ID |
| HLA-A |
| HLA-B |
| $PI^{A1}$(HPA-1a) |
| $PI^{A2}$(HPA-1b) |
| $Pen^{a}$(HPA-4a) |
| $Pen^{b}$(HPA-4b) |
| $Bak^{a}$(HPA-3a) |
| $Bak^{b}$(HPA-3b) |
| $Br^{a}$(HPA-5b) |
| $Br^{b}$(HPA-5a) |
| $Ca^{a}$(HPA-6b) |
| $Ca^{b}$(HPA-6a) |
| $Gov^{a}$(HPA-15b) |
| $Gov^{b}$(HPA-15a) |
| $Max^{a}$(HPA-9b) |
| $Max^{b}$(HPA-9a) |
| $Ko^{a}$(HPA-2b) |
| $Ko^{b}$(HPA-2a) |

# Typical results of positive NAIT

- Maternal plasma crossmatched with paternal platelets are usually incompatible
- HPA typings will confirm antigen incompatibility, typically seen with HPA-1a

# Neonatal Alloimmune Thrombocytopenia

- IRL recommends transfusing random platelets to “soak up” whatever finite number of maternal antibody is circulating

- If requested, HPA-1a negative donors can try to be reached for donation

# IRL's Role

Received a NAIT workup from hospital A: Baby has a low platelet count

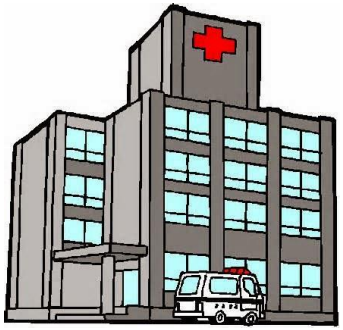
- Maternal sample was nonreactive with two test methods when tested for platelet antibodies
- Both maternal and paternal samples negative for Platelet bound IgG
- Maternal sample (group O) compatible with paternal platelets (group B)
- No HPA-1a typings done since mom was compatible with dad

*"The results of these tests do not provide serologic evidence of neonatal immune thrombocytopenia"*

# IRL's Role

Next day, IRL received notification from Hospital A that Baby was still doing poorly:

- Baby not getting a very good bump from plt transfusions
- Baby currently receiving IVIG
- Were transfusing group A and AB platelets, we recommended transfusing group O



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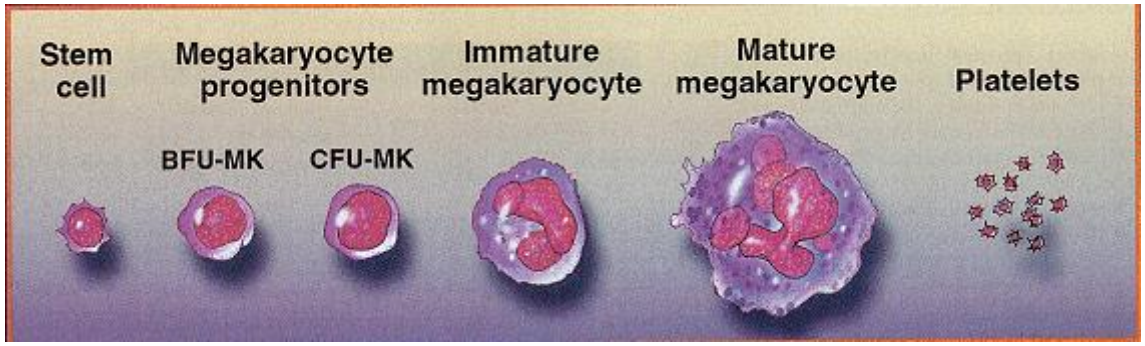
# IRL's Role

Notified that Baby had a bone marrow aspirate done and no megakaryocytes were found

- If megakaryocyte line is only one effected, gives evidence that issue is not an immunologic problem



# IRL's Role

- The following day, IRL is notified that Baby has been transferred to another hospital "B"
  - Baby's current platelet count on day 2 is 17,000
  - New facility is requesting HPA-1a negative platelets, which CBC does not have
  - CBC contacted New York Blood Center, no HPA-1a negative platelets
  - A call list of donors was created to try and get a product
  - Also notified that megakaryocytes were observed in BM….

# Side Note

- Why is it so difficult to locate HPA-1a negative donors
  - Being designated with “a”, HPA-1a comprises 98% of all donors
  - Leaving 2% of donors compatible
  - Currently, CBC only tests about 50% of donors….
  - Effectively making it twice as hard to find an already difficult to find unit

# IRL's Role

- Hospital B calls inquiring about the availability of HPA-1a negative platelets – Baby's platelet count not rising after numerous transfusions
  - Unable to find available donors
- Interested in getting HLA and HPA testing on Mother, Father and Baby
  - Requires samples to be sent to New York Blood Center, 2 separate labs

# IRL's Role

- Breakdown of platelet counts:
  - Day two: plt count 17,000 – two plt transfusions
  - Day three: plt count 51,000
  - Day four plt count 5,000 – one plt transfusion
- Received several more plt transfusions throughout the next two days
- Platelet counts hovered between 30K and 50K

# IRL's Role

HPA Results

| | 1a | 1b | 2a | 2b | 3a | 3b | 4a | 4b | 5a | 5b | 6a | 6b | 7a | 7b | 8a | 8b | 9a | 9b | 11a | 11b | 15a | 15b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pat | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + |
| Mat | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 |
| Baby | + | + | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + |

HLA Results

| | HLA-A | HLA-B |
|---|---|---|
| Maternal | 02:03 | 07:08 |
| Paternal | 01:24 | 08:35 |
| Baby | 01:03 | 07:08 |

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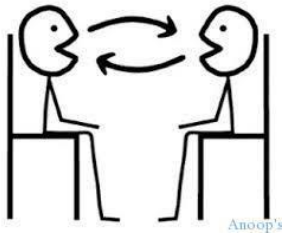


# IRL's Role

- Once results were received, consultation with hospital B occurred
  - Just because mom is negative for HPA-1a, doesn't mean she has antibody: test was negative and mom was compatible with dad's platelet
  - But….could be a low titer antibody that is undetectable with our methods: will continue to look for HPA-1a negative donors
  - Hospital B was going to send additional sample out to another facility for further testing

# IRL's Role

- Hospital B notified the lab that the patient has been discharged with a 66,000 plt count
  - The patient's platelet counts remained stable one week later at 73,000
  - The patient returned one month later with a platelet count of 673,000
  - No HPA-1a negative platelets were ever transfused
  - No HLA matched platelets were ever requested

# Testing from Outside Facility

- Genetic testing was performed on Baby by outside facility
  - A heterozygous variant was observed in gene ITGB3, which codes for cell surface proteins on platelets
  - This ITGB3 variant, p.Leu59Pro, has been associated with platelet-type bleeding disorder 16:
    - Premature coronary heart disease
    - Myocardial infarction
    - Hip fracture
  - The ITGB3 variant has also been associated with <u>Neonatal Alloimmune Thrombocytopenia</u>

# An Interesting Find



Published in final edited form as:
*Transfusion*. 2013 June ; 53(6): 1309–1318. doi:10.1111/j.1537-2995.2012.03903.x.

**Prevalence and clinical significance of low-avidity HPA-1a antibodies in women exposed to HPA-1a during pregnancy**

"Findings confirm previous reports that low-avidity HPA-1a antibodies can cause NAIT"

# Conclusion

- Testing on maternal sample by two methods showed no antibodies present and XM compatible with paternal plts
  - HPA typing did show mismatch in HPA-1a
- Although no antibodies were detected, that does not supersede Baby's clinical presentation – low titer antibody may not show up
  - Would be worth seeing if HPA-1a negative plts had any better effect
- Platelet-type bleeding disorder 16: a disorder in platelet production

# References


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# QUESTIONS?

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