NEONATAL ALLOIMMUNE THROMBOCYTOPENIA
A severe case of neonatal alloimmune thrombocytopenia requiring early laboratory intervention

Course # DL-998

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1.0 CE
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Abstract: (Case Study)
The patient presents as a 26 year-old gravida 3 para1 female. She has a history of neonatal alloimmune thrombocytopenia (NAIT) in a previous pregnancy and now has another affected pregnancy. NAIT is a little-known disease that poses a major problem for both the fetus and the physician. In NAIT the mother produces antibodies against fetal platelet antigens. These antibodies can cross the placenta as early as 17 weeks of gestation. Consequences of the antibody passage can be minimal or as serious as intracranial hemorrhage (ICH) that could lead to primary hemorrhagic morbidity. Most cases are not diagnosed until the index case is discovered, as in this patient’s losing a fetus at 36 weeks. During the present pregnancy fetal platelet transfusions were performed and the mother received intravenous immune globulin (IVIG)/prednisone injections every week. The baby was delivered via Caesarian section and after a four week hospital stay was discharged. No evidence of ICH was shown and no additional transfusions were necessary.

Objectives:
After completing this course the participant will be able to:
1. describe NAIT and its prevalence
2. outline the platelet antigens most frequently involved in Caucasians and Asians
3. discuss the laboratory tests involved in diagnosis and treatment of NAIT
4. outline a treatment method in controlling a severe case of NAIT
5. describe methods used to diagnose possible NAIT in a fetus
6. discuss methods of preventing manifestations of NAIT in an at-risk fetus

Key words
NAIT/NATP, FMAIT: Neonatal alloimmune thrombocytopenia (aka feto-maternal alloimmune thrombocytopenia)
NT/NNT: neonatal thrombocytopenia
ICH: intracranial hemorrhage
HDN: hemolytic disease of the newborn
HPA: human platelet antigen
IVIG: intravenous immunoglobulin
GPIII: platelet glycoprotein
GPIV: CD36, thrombospondin receptor
HLA: human leukocyte antigen  
Gravida: the number of times the mother has been pregnant, including the current pregnancy  
Para: the number of live births

**History**

The patient is a 26-year-old white, non-Hispanic female (gravida 3, para 1) whose first pregnancy was unaffected but presented with a decreased fetal movement at 36 weeks of gestation in her second. After an ultrasound was performed, massive intracranial hemorrhage was noted in the fetus and as a result neonatal alloimmune thrombocytopenia (NAIT) was diagnosed. Both she and her husband had platelet genotyping; she is HPA-1b homozygous, while he is HPA 1a/1b. She now presents with another pregnancy. An amniocentesis was performed at 18 weeks for fetal platelet genotyping. Results showed this pregnancy to be at risk for NAIT—i.e., the fetus’ platelet genotype is HPA 1a/1b.

**Disease Mechanism**

Maternal thrombocytopenia is common during pregnancy, but fetal thrombocytopenia is not (1). NAIT, by definition, is a decreased platelet count in the fetus caused by maternal antibodies against fetal platelets that bear paternal antigens (2). Sensitized fetal platelets will be destroyed via the fetal monocyte-phagocyte pathway. Incidence of affected infants varies from 1 in 1000 to 1 in 5000 (3). NAIT is often a diagnosis of exclusion; all other possibilities having been ruled out. Similar to ABO-HDN, NAIT may affect the first pregnancy. Maternal IgG anti-platelet antibodies can cross the placenta as early as 17 weeks (3).

Expression of human platelet antigens (HPA) is genetically determined and is associated with platelet membrane glycoproteins (GP). To date 26 specific human platelet antigens have been identified. Fourteen have been grouped into seven biallelic systems, as follows:

<table>
<thead>
<tr>
<th>Human Platelet Antigen Systems *</th>
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<tr>
<td><strong>System</strong></td>
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<td>HPA-1</td>
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Twelve antigens do not, as yet, have antithetical alleles. In Caucasians NAIT is primarily caused by antibodies to antigens in the HPA-1 system, accounting for 80-90% of cases, or HPA-5 system, accounting for 5-15% of cases. In Asians the HPA-4 system is more frequent, while NAIT associated with the HPA-1 system almost never occurs (4). Out of the twenty-six platelet-specific antigens (5) that have been characterized, twenty-one of these result from a single amino acid difference (6) caused by a single nucleotide substitution in the coding gene.

HPA-1 is part of the platelet surface glycoprotein (GP) IIIa, which complexes with GP IIb to form a heterodimer on the platelet membrane. HPA-1a is expressed by most of the population (98%) while HPA-1b is expressed when a point mutation occurs, causing a leucine to proline substitution (2). HPA-5a, formerly named Br, occurs in 99% of the population.

Another cause of NAIT may occur in women with deficiency of platelet CD36 (glycoprotein IV). This deficiency occurs in 3-5% of persons of Asian or African ancestry. Isoimmunization against the CD36 marker occurs in some individuals. In a study by Curtis et al. this was identified as a cause of NAIT in five infants (7). These mothers all exhibited a lack of detectable GPIV on their platelets and transplacental immunization occurred, much the same as the other HPA antibodies (6). Not all cases of feto-maternal incompatibility result in antibody production (8,9) though the reason is unknown.

**Disease**

Most of the cases of NAIT are diagnosed after birth. There may be no symptoms in mildly affected infants. Infants with severe thrombocytopenia may present with petechiae or a cephalohematoma at birth. Evidence of intracranial hemorrhage (ICH) occurs in 10-20% of affected newborns. In these severe cases of NAIT, serious bleeding diathesis, death of the fetus or neonate can occur in 10% of the infants and neurological sequelae occur in 20% (6). Primary hemorrhagic morbidity and mortality cases are often found to be a result from NAIT. ICH occurs mainly during delivery, due to the trauma of delivery, or immediately after, but could occur spontaneously according to recent investigations (10). In utero ICH usually occurs between 30-35 weeks (10), but can occur much earlier if maternal antibodies have a high titer. Severity of disease is also associated with Human Leukocyte Antigen (HLA) DRB3*0101 according to a recent study in Norway (11). Subsequent pregnancies may be affected earlier each time, due to increased antiplatelet antibody titer in the mother, posing a problem for physicians because antenatal platelet screening is not routinely ordered.

Other causes of fetal thrombocytopenia include:

- **Infection/sepsis**
  - Congenital TORCH infection
    - *Toxoplasma gondii*
    - Rubella virus
    - Cytomegalovirus
    - Herpes simplex virus
  - Other infections
    - Hepatitis B
    - *Treponema pallidum*
    - Varicella-zoster virus
    - HIV
    - Parvovirus B19
Perinatal infection with Escherichia coli, *Haemophilus influenzae*, group B *Streptococcus (S. agalactiae)*

- Disseminated intravascular coagulation
- Maternal autoimmune thrombocytopenia with transplacental passage of antibodies
  - Immune thrombocytopenic purpura
  - Systemic lupus erythematosus
- Maternal exposure to antiplatelet medications
- Bone marrow abnormalities or inherited thrombocytopenias

**Laboratory Role**

Unlike other forms of HDN, no prophylaxis, such as RhoGAM in Rh-HDN, exists for platelet antibodies, nor is there an agreed upon antenatal treatment program. Identification of the index case is crucial to the successful gestation of another child. Among the papers researched, a consensus was reached that screening for NAIT would not be cost effective in the general population, increasing the importance of identifying the index case.

If NAIT is suspected, a fetal platelet count may be obtained by percutaneous umbilical cord vein sampling, though it is not without risk. Immunization could occur due to the sampling itself or could induce fetal bleeding. During delivery, the scalp vein may be used, presuming the fetus is presenting in the correct position. Demonstration of circulating maternal antibody would be the least invasive way to confirm a possible case. However, presence of maternal antibodies does not correlate with clinical symptoms in all cases (1). Additionally, in a study by de Moerloose, only one-third of cases demonstrated antibodies. Platelet genotyping should be performed on the mother and father when fetal sampling has not been done. If the parents are homozygous for opposite alleles, the fetus will be an obligate heterozygote with NAIT as a possibility. If the father is heterozygous for an allele the mother lacks, then the fetus will only have a 50% chance of being affected. In these cases, confirmation of the offending antigen can only be determined by fetal platelet genotyping.

Typing should be performed on platelet antigens 1-5. Some HLA antigens should be tested due to an increasing number of cases involving antigens other than the HPA system (6). Platelet tests are performed only in specialty labs, such as the Blood Center of Wisconsin. Platelet genotyping may be performed on DNA isolated from fetal leukocytes, amniotic fluid, cultured amniocytes, or chorionic villi. DNA is then amplified by PCR with allele specific primer extension with analysis done by Luminex bead microscopy (5).

Antibody screens for platelets are done by flow cytometry with serum samples. The sample is tested against group O donor platelets and can detect autoantibodies, some HLA antibodies, and HPA 1-5 antibodies. *In vitro* antibody binding is detected by polyclonal antibodies to human IgM and IgG (5). Once platelets have been characterized, platelet transfusions for the fetus may be prepared. Maternal platelets would be the best choice (as long as she is blood type O) for intrauterine transfusion. In this specific case, maternal platelets could not be used due to the problems with the collection. Due to the low prevalence of HPA-1b homozygous units, transfusion had to be delayed to search for units.

**Treatment**

In this case, maternal platelet antibody screen was performed during the first affected pregnancy. Maternal platelet genotyping was determined to be HPA-1b homozygous, prompting paternal genotyping. The paternal specimen was heterozygous for HPA-1a/1b.
In the subsequent pregnancy, she was placed in a high-risk category because of the previous pregnancy with ICH. At 18 weeks an amniocentesis was performed to collect fluid to send to the Blood Center of Wisconsin for fetal platelet typing, AF-AFP determination, and karyotyping. Fetal platelets typed out heterozygous for HPA-1 (i.e., 1a/1b), indicating that the antibody must be anti-HPA-1a. An aggressive treatment was then undertaken for the duration of the pregnancy. IVIG was administered 1g/kg starting at 25 weeks. An ultrasound was performed at 29 weeks, along with a fetal platelet transfusion, due to a critically low platelet count of 13x10⁹/L. After fetal platelet transfusion (53mL), intensification of treatment was suggested; the platelet count was still below 30x10⁹/L. Treatment was increased to 1g/kg of IVIG twice weekly and prednisone was added (1mg/kg) daily. Platelet transfusion was again attempted at 33 weeks; however the fetus moved during the procedure and received only 20 mL of platelets. Caesarian section was performed the next day due to dropping maternal platelet levels and no antigen negative platelet units readily available. The child required no platelet transfusions and was the appropriate size for his gestational age. At birth the platelet count was 226x10⁹/L and showed no significant drop during the four week hospital stay. Preemptive antibiotic regimens were initiated for the first 72 hours of life due to a high sepsis risk, though no sepsis presented. IVIG was administered on day two of life. Respiratory acidosis was present for a short time, but the child was weaned to room air by day eleven. Antibody screens for both the mother and child remained negative. Phototherapy was needed for 10 days and a small right choroid plexus cyst was noted. Respiratory acidosis, phototherapy, and preemptive sepsis treatment are normal for premature infants, and are not specific to NAIT symptoms. The choroid plexus cyst occurs spontaneously in 1% of newborns with no known effects on learning or development. The only association known is to trisomy 18, Edward’s disease, which is not conducive to life (4). There was no mention of a trisomy in the patient data, so it can be assumed this disease is not present.

Conclusion:
Despite an early transplacental cross of maternal antibodies, aggressive treatment proved to be successful in preventing a second child succumbing to NAIT. If the mother decides to have another child, strict monitoring is necessary to prevent antibodies crossing the placenta earlier. This case demonstrates the importance of correct clinical diagnosis of NAIT. The disease is not specific to any HPA antigen in particular, though it occurs most frequently on HPA-1 in Caucasians and HPA-4 in Asians. Prenatal platelet typing may become routine based on an estimate by researchers that it could save the US $2.2 million annually (11). Monitoring should take place with known affected mothers and couples should be counseled on the complicated nature of this disease in order for them to take appropriate measures if they decide to conceive again.
Bibliography
REVIEW QUESTIONS
Course #DL 998
Choose the one best answer:

1. NAIT is caused by
   a. decreased platelet count in the mother.
   b. destruction of platelets in the fetus by maternal antibodies.
   c. incompatibility between red cell antigens in the mother and fetus.
   d. mother being HPA 1a/1b and the father being 1a/1a.

2. Which of the following situations could cause NAIT in a fetus?
   a. mother is HPA 1a/1b and the father is HPA 1b/1b
   b. mother is HPA 1a/1b and the father is HPA 1a/1b
   c. mother is HPA 1b/1b and the father is HPA 1a/1b
   d. infant’s platelets are HPA 1a/1a and the mother is HPA 1a/1b

3. Signs characteristic of NAIT in the infant include all the following except
   a. intracranial hemorrhage
   b. petechiae
   c. decreased platelet count
   d. jaundice

4. Laboratory tests for NAIT include
   a. platelet count in the mother
   b. platelet antibodies in the fetus
   c. platelet count in the fetus
   d. RBC count in the fetus

5. An Asian infant shows signs of NAIT. Which of the following HPA systems should be
   investigated first as the most likely cause?
   a. HPA-1
   b. HPA-2
   c. HPA-4
   d. HPA-5

6. Treatment of NAIT includes
   a. intrauterine transfusion using mother’s platelets.
   b. intrauterine transfusion using father’s platelets.
   c. intrauterine packed RBC transfusion.
   d. removal of antibodies from infant’s blood.

7. An infant is at risk for NAIT
   a. only after sensitization of the mother in the first pregnancy.
   b. when the mother is CD36 negative and the father is CD36 positive.
   c. when the mother is Rh negative and the father is Rh positive.
   d. only when the father is homozygous for a platelet antigen the mother lacks.
8. Other causes of decreased platelet count in the fetus mentioned in the article include all the following except
   a. TORCH infections
   b. HIV
   c. Hepatitis B
   d. Measles

9. Screening for platelet antibodies is usually performed
   a. in hospital laboratories.
   b. in specialty laboratories.
   c. by PCR.
   d. on the infant’s serum.

10. In Caucasians the platelet HPA systems most likely involved are
    a. HPA-1 and HPA-5
    b. HPA-3 and HPA-5
    c. HPA-1 and HPA-4
    d. HPA-2 and HPA-3