Platelet Antibodies

Objectives:

1. Name three types of antigens expressed on the surface of platelets.
2. Discuss immune platelet refractoriness, including antibodies that cause refractoriness and transfusion options.
3. Describe two conditions caused by antibodies to human platelet antigens (HPA).

Quick lesson:

Antigens expressed on platelets		
System	**Description**	**Antibodies to these antigens…**
ABO	Expressed on platelets, as on most body tissues	contribute to platelet refractoriness
HLA	**Human Leukocyte Antigen,** Class I, A & B antigens	cause immune platelet refractoriness
HPA	**Human Platelet Antigens**: antigens expressed on platelet glycoproteins	may cause: • Post transfusion purpura (PTP) • Fetal/neonatal alloimmune thrombocytopenia (FNAIT)

Platelet Refractoriness:

Platelet refractoriness is a less-than-expected increase in platelet count following platelet transfusion. There are calculations (example: CCI, or Corrected Count Increment), that include platelet count of the product transfused and body mass of the recipient, used to determine how much a platelet count should increase following transfusion. Typically, two consecutive poor responses to platelet transfusion define platelet refractoriness.

Causes:

Most cases of platelet refractoriness are caused by nonimmune factors like bleeding, disease state, drugs, fever, etc. Antibodies to antigens on platelets cause immune platelet refractoriness.

Immune-Mediated Platelet Refractoriness:

Antibodies to HLA Class I antigens are the most common cause of IMMUNE platelet refractoriness.

Platelet Refractoriness: (continued)

Laboratory testing:

- **Platelet antibody screen**: by solid phase red cell adherence assay (SPRCA) or ELISA (ELISA testing can differentiate HLA from HPA antibody reactivity). Can detect incompatibility with platelets or glycoproteins that indicate patient has made antibody.
- **Platelet crossmatching**: typically SPRCA methodology – allows transfusion of platelet products compatible with patient's plasma.

Transfusion Options:

Transfusion options for patients with platelet refractoriness				
Product	**Description**	**Benefits**	**Risks**	**Special Considerations**
Crossmatched platelets	Platelets serologically compatible with patient's plasma (usually SPRCA)	Usually available quickly	• Possible exposure to new/different foreign antigens that can elicit further alloimmunization	All crossmatched or HLA-matched platelet products must be ***irradiated*** to prevent Transfusion-Associated-Graft-vs-Host Disease
HLA-matched platelets	Platelets from donors chosen because they closely match patient's HLA Class I A & B antigens	Perfectly matched products don't expose patient to new foreign antigens	• Requires testing to determine patient HLA typing • Requires large donor pool to accommodate many patients, especially those with less common HLA types • There are different HLA match grades; some better than others • May take time to acquire	
	Platelets chosen for their HLA Class I antigens, avoiding the specificities of patient's antibodies	Easier to procure than products that are an exact HLA match	• Requires testing to determine patient HLA typing & identification of patient HLA antibodies • Requires large donor pool to accommodate many patients, especially those with multiple HLA antibody specificities • May take time to acquire • Possible exposure to new/different foreign antigens that can elicit further alloimmunization	

Case Study: Platelet Refractoriness

Patient sample submitted to lab for platelet antibody screen as physician suspects immune-mediated platelet refractoriness. Patient is undergoing chemotherapy for leukemia and has received multiple platelet products.

Platelet antibody screen: test patient plasma against a panel of 8 donor platelets by SPRCA.

Results:

Platelet antibody screen positive: Patient plasma reactive with 5/8 random platelets

What might be the easiest/fastest transfusion option for this patient?

a. Crossmatched platelets
b. HLA-matched platelets, exact HLA match
c. HLA-selected platelets, avoiding patient's HLA antibodies

Answer: a. Crossmatched platelets

Explanation:

- 3 of 8 random platelets are compatible with the patient's plasma. That is, compatible products are fairly easy to find within blood center's inventory.
- HLA-matching would require first testing the patient's sample to determine patient's HLA type. Then, searching for HLA-matched donors would be required. This would take some time.
- Selecting donor platelets that avoid the patient's HLA antibodies would also require identification of the HLA antibody specificities in the patient's plasma, then searching for acceptable donors. Again, this would take some time.

FNAIT: Fetal & Neonatal Alloimmune Thrombocytopenia (FNAIT):

A pregnant woman develops antibodies (against HPA antigen, usually) that cross the placenta & destroy fetal/neonatal platelets causing thrombocytopenia and/or bleeding in fetus or neonate. In this way, it is similar to hemolytic disease of the fetus/newborn (HDFN).

Comparison of FNAIT and HDFN		
	FNAIT	**HDFN**
Etiology	Mom makes antibodies to antigen on fetal platelets. Antibodies cross placenta and destroy fetal platelets.	Mom makes antibodies to antigen on fetal RBCs. Antibodies cross placenta and destroy fetal RBCs.

Common implicated antibody	• HPA antibodies
	• ~80% of cases due to **anti-HPA-1a**
	  ○ HPA-1a antigen is expressed on the platelets of about 98% of Caucasians; women who are HPA-1a-negative are at risk
		• Many potentially clinically significant RBC antibodies
		• Prior to widespread use of Rh immune globulin (RhIg), anti-D was most commonly implicated
		  ○ D antigen is expressed on RBCs of about 85% of population; D-negative women at risk prior to RhIg
		• ABO antibodies are now the most common cause of HDFN, though ABO HDFN is often mild

Most severe symptom	Intracranial hemorrhage (ICH)	Hydrops fetalis, stillborn
Prevalence	1:1000 births 1:10,000 severe (ICH)	Prior to RhIg, 7% of pregnancies affected by anti-D HDFN
Affects first pregnancy	Yes, platelet antigens are developed early in gestation, most ICH occur prenatally	No, usually birth is immunizing event; antibody affects subsequent pregnancies with D+ fetus
Screening	None	All pregnant women are screened for D typing
Prophylaxis	None	D-negative women receive RhIg to prevent alloimmunization
Diagnosis	Usually made if sister has a history of FNAIT, or after birth of affected neonate	Diagnosed during pregnancy, as women are screened

FNAIT Laboratory testing

- Requires sample from mom & dad (not baby)
- Tests included
  - Maternal plasma: antibody screen
  - Maternal plasma: crossmatch against paternal platelets
  - Maternal & paternal platelets:
    - Platelet bound IgG (rules out autoimmune process)
    - HPA-1a typing (usually genomic testing)

FNAIT Common laboratory results:

	Antibody screen	Platelet-bound IgG	Crossmatch with paternal platelets	HPA-1a typing	Interpretation
Maternal sample	Positive	Negative	Positive	Negative	FNAIT due to anti-HPA-1a
Paternal sample	Not tested	Negative	Not tested	Positive	

FNAIT Treatment:

Prenatally: only if woman has a history of delivering a baby with FNAIT or has a sister with history

- IVIG, steroids (no consensus), monitoring

Neonate:

- Upon discovery of thrombocytopenia, scan for ICH
- Platelet transfusion options:
  - Ideally, antigen negative (HPA-1a-negative), but random platelets are also effective
  - Transfusion of maternal platelets possible, but must be washed (to remove antibody) and irradiated
- IVIG

Case study: FNAIT

A sample is submitted for an FNAIT workup. The neonate is a full-term, otherwise healthy male with petechiae and ecchymosis. Here are the results of the workup:

	Antibody screen	Platelet-bound IgG	Crossmatch with paternal platelets	HPA-1a typing	Interpretation
Maternal sample	Positive	Negative	Positive	HPA-1b/b	FNAIT due to anti-HPA-1a
Paternal sample	Not tested	Negative	Not tested	HPA-1a/b	

The infant is transfused with HPA-1a-negative platelets, and recovers well.

What is the probability of FNAIT in a subsequent pregnancy?

a. 100% of future pregnancies may be affected
b. 50% of future pregnancies may be affected
c. Unknown

Answer: b

Explanation: While mom is HPA-1a-negative, and has made anti-HPA-1a, dad is HPA-1a/b, meaning there is only a 50% chance that offspring will inherit HPA-1a.

PTP: Post-Transfusion Purpura

Definition:

- A rare, delayed transfusion reaction characterized by sudden onset of self-limiting thrombocytopenia/ bleeding episodes
- Occurs 5-10 days following transfusion (usually transfusion of RBCs)
- Most implicated antibody: **anti-HPA-1a**
  - Anamnestic response- often affects multiparous HPA-1a-negative females
  - ****Strangely, both transfused (HPA-1a+) and autologous (HPA-1a-) platelets are destroyed**

Diagnosis:

- Clinical picture
  - Post-transfusion
  - Severe, sudden drop in platelets
- Identification of HPA antibody in patient plasma
- Genotyping (Patient antigen negative for corresponding antibody)

Treatment:

- IVIg (with or without steroids)
- Plasma exchange to remove antibody
- Splenectomy
- Platelet transfusions: only if bleeding (remember antigen negative platelets are destroyed as well)
  - Give antigen negative platelets & RBCs if transfusion indicated (HPA-1a-negative platelets and RBCs)
  - Give washed RBCs (theoretically washing away residual platelets)

Assessing Understanding:

1. **A patient's plasma is found to contain HLA antibody. Which of the following conditions is most often associated with HLA antibodies?**
    a. FNAIT
    b. PTP
    c. Immune platelet refractoriness
    d. All of the above

2. **Why must crossmatched and HLA-matched platelet products be irradiated prior to transfusion?**
    a. To destroy HLA antigens
    b. To prevent platelet refractoriness
    c. To prevent TA-GVHD
    d. To prevent HPA alloimmunization

3. **Which HPA antibody is implicated in most FNAIT and PTP cases?**
    a. Anti-HPA-1a
    b. Anti-HPA-1b
    c. Anti-HPA-5b
    d. Anti-HPA-15b

Answers: 1. c; **2**. c; **3**. a