PNH—An Overview

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PATHOPHYSIOLOGY OF PAROXYSMAL NOCTURNAL HEMOGLOGINURIA

Much Madness is divinest Sense
To a discerning Eye
Emily Dickinson (1830-1886)
#435

First descriptions of PNH
William Gull-1866
Paul Strübing-1882

Case Presentation

- A 31 years old female presented to an ER with complaints of fever and dark urine.
- Hgb 3.8 gm/dl; Hct 12%, WBC 4,100/µl; plt count 171,000; LDH 1872 (ULN 240 IU/L); reticulocyte count 11.5%; haptoglobin <6 mg/dl.
- A diagnostic test was done
Flow Cytometric Diagnosis of PNH

Case Presentation (continued)

- Peripheral blood: mild to moderate anisocytosis, minimal poikilocytosis; rare RBC fragments; no spherocytes; no abnormal platelet or WBC morphology.
- Bone marrow aspirate: normal RBC and WBC maturation with erythroid predominance.
- Bone marrow biopsy: hypocellular for age (40%) with erythroid islands mixed with myeloid cells.
- Sequencing of PIGA cDNA: 14 bp deletion in exon 2 introduced a premature stop codon.
- Treatment with eculizumab was initiated with marked improvement in disease signs and symptoms.
- The patient had an identical twin and underwent hematopoietic stem cell transplantation.
- After 3 years of follow-up, she has no evidence of PNH.

What Is PNH?

(more than a hemolytic anemia)

- PNH is a consequence of nonmalignant clonal expansion of one or several hematopoietic stem cells that have acquired a somatic mutation of PIGA.
- Progeny of affected stem cells are deficient in all glycosyl phosphatidylinositol-anchored proteins (GPI-APs) that are normally expressed on HSCs.
- Clinical manifestations: hemolytic anemia, thrombophilia, bone marrow failure.
Epidemiology

- Men and women are affected equally
- Peaks in the 4th decade but occurs in all age groups
- Found throughout the world
  - Prevalence may be increased in regions where the incidence of aplastic anemia is above normal
- Prevalence of clinical PNH: 3-6/million population
  - 900-1,800 cases in the US

PNH—The Genetic Basis:
The GPI-anchor is complex

Pathophysiology of PNH Is Known
Mutations in PNH Cause **Loss-of-Function of PIGA**

Characteristics of PNH

- PNH is **not a binary process**
- The clinical manifestations are determined primarily by:
  - The size of the PNH clone
    - The peripheral blood of patients is a mosaic of normal and abnormal cells
  - The degree of deficiency of GPI-APs
    - Some cells are **completely deficient** in GPI-APs while others are **partially deficient**

Phenotypic Mosaicism Based on Flow Cytometry


Endo et al. *Blood* 1996;87:2546-2557
High-Resolution Flow Cytometry for Diagnosis of PNH

RBCs

PMNs

Flow Cytometric Diagnosis of PNH

Normal Control

Patient

RBCs

PMNs

Clinical Manifestations are Determined by Clone Size and Phenotype

Patient with high percentage of type III cells: high-grade hemolysis

Patient with high percentage of type II cells but low percentage of type III cells: minimal hemolysis

Patient with low percentage of type III cells: minimal hemolysis
Selection of *PIG-A* Mutant, GPI-AP Deficient Stem Cells in Aplastic Anemia

Clonal Expansion of *PIG-A* Mutant, GPI-AP Deficient Stem Cells

Natural Selection of PNH Hematopoietic Stem Cell
Who Should be Screened for PNH?

- Patients with a history of episodic hemoglobinuria
- Patients with evidence of non-spherocytic, Coombs’ negative intravascular hemolysis (must have abnormally high serum LDH)
- Patients with aplastic anemia (screen at diagnosis and once yearly even in the absence of intravascular hemolysis)
- Patients with refractory anemia-MDS
- Patients with venous thrombosis involving unusual sites (usually have evidence of intravascular hemolysis)
  - Budd-Chiari syndrome
  - Other intra-abdominal sites
  - Cerebral veins
  - Dermal veins

PNH—More Than a Hemolytic Anemia

Basic evaluation for PNH

- Flow cytometric evidence of a population of peripheral blood cells (erythrocytes, granulocytes, or preferably both) partially or completely deficient in multiple glycosyl phosphatidylinositol-anchored proteins (GPI-APs).
- Complete blood count, reticulocyte count, serum concentration of lactate dehydrogenase (LDH), bilirubin (fractionated) and haptoglobin, iron stores
- Bone marrow aspirate, biopsy, and cytogenetics
### Signs and Symptoms of PNH

- **Constitutional (due to intravascular hemolysis)**
  - Fatigue, lethargy, asthenia, loss of sense of well being
- **Bone Marrow Failure**
  - Excessive bleeding or bruising secondary to thrombocytopenia (low platelets)
  - Infections due to low neutrophil count
  - Shortness of breath, fatigue due to anemia
- **Thrombophilia (clotting)**
  - Swelling of leg or arm
  - Abdominal pain
  - Headache

### Key Laboratory Test

- **Bone Marrow Function**
  - CBC (complete blood count) monitors white blood cells, red blood cells (hemoglobin and hematocrit) and platelets
  - Reticulocyte count monitors red cell production
- **Hemolysis**
  - LDH (lactate dehydrogenase)
- **Iron studies (iron deficiency due to hemoglobinuria)**
- **Flow cytometry**
  - Monitors clone size (yearly unless some change is noted)
How Do I Know If I Have a Blood Clot?

- Deep venous thrombosis
  - Swelling, redness, pain in an extremity (arm or leg)
- Dermal vein thrombosis
  - Pain, redness, swelling involving the skin (usually the arm, but other sites can be involved)
- Splanchnic (abdominal vein thrombosis)
  - Pain, cramping, nausea, vomiting
  - Budd-Chiari syndrome: Abdominal swelling (ascites), jaundice
- Cerebral vein
  - Headache, nausea, vomiting, photophobia, cognitive dysfunction (confusion, speech or memory problems)

Diagnostic Tests for Blood Clots

- Deep venous thrombosis
  - Doppler ultrasound
- Dermal vein thrombosis
  - Visual inspection of affected site
- Splanchnic vein thrombosis
  - Imaging studies (ultrasound, CT scan, MRI scan)
- Cerebral vein thrombosis
  - Imaging studies (CT scan, MRI scan)

Management of the Thrombophilia of PNH

- Prophylaxis
  - Recommended for patients with clone size ≥ 50% who are not being treated with eculizumab
  - Not recommended for patients being treated with eculizumab who have never had thrombosis
- For patients with a history of thrombosis who start treatment with eculizumab
  - Continue anticoagulation
PNH and Aplastic Anemia

• There is a close association between PNH and bone marrow failure syndromes, particularly aplastic anemia.
• The immune attack on the bone marrow that underlies aplastic anemia is thought to be selected for PIGA-mutant GPI-AP deficient HSCs.
• The basis of the selection is speculative.

Bone Marrow Biopsy

• A young patient with PNH
• The photographs are from adjacent fields in the same biopsy specimen

Stephen Richards, Leeds, UK
PNH and Bone Marrow Failure

- **PNH/Aplastic anemia**
  - Should be managed the same way as AA without PNH
  - Immunosuppressive therapy does not affect the PNH clone
- **PNH/MDS**
  - Some studies suggest that the finding of PNH cells in patients with MDS predicts a response to immunosuppressive therapy

**Targeted Therapy for PNH**
**Alternative Pathway of Complement Activation on Erythrocytes**

*C3 convertase: C3b-Bb-P

*C5 convertase: C3b-Bb-C3b-P

**Membrane Attack Complex**

C5b - 9n

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**Generation of C3 Opsonins* on Erythrocytes**

*C3 opsonins, iC3b and C3dg, target RBCs for destruction by reticuloendothelial cells expressing complement receptors:

- CR1
- CR3

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**Eculizumab Treatment Alters the Natural History of PNH**

A

H17/3E7

H2O + C3b

PNH Untreated

PNH + H17/3E7

PNH + eculizumab

B

H17/3E7

No hemolysis or C3 opsonization

PNH + H17/3E7
What Does Eculizumab Do?

- Blocks Intravascular Hemolysis
- Reduces transfusion requirements
- Prolongs transfusion interval
- Ameliorates symptoms associated with chronic and acute intravascular hemolysis
  - Malaise, lethargy, fatigue
  - Abdominal pain, dysphagia, male impotence
- In many (but not all cases), transforms PNH into a minimally symptomatic disease

What Doesn’t Eculizumab Do?

- Block Extravascular Hemolysis
  - Mediated by complement opsonization of RBCs
- Completely eliminate transfusion requirements in all patients
- Eliminate anemia
- Affect the underlying process
  - Clonal hematopoiesis
  - Bone marrow failure
- Effective therapy because PNH is not a malignant clonal disease

What It Probably Does

- Ameliorate the thrombophilia of PNH
Who Clearly Benefits From Eculizumab?

- Patients with Classic PNH
  - Large PNH type III clone (usually >90% GPI-AP deficient granulocytes)
  - Symptoms that are due to chronic intravascular hemolysis (regardless of transfusion requirements)

Management of the Anemia of PNH

- Treatment Options (empirical and supportive)
  - Corticosteroids
  - Androgenic Steroids
  - Transfusions
  - Iron Replacement
  - Erythropoietin Supplementation

Hematopoietic Stem Cell Transplant for PNH
Indications Before Eculizumab

- **Bone marrow failure**
  - Decision on transplant based on aplastic anemia or less commonly MDS

- **Major complication of Classic PNH**
  - Refractory, transfusion-dependent hemolytic anemia
  - Recurrent, life-threatening thromboembolic disease

  *Parker et al, Blood 2005*

Indications After Eculizumab

- **Bone marrow failure**
  - Decision on transplant based on aplastic anemia or less commonly MDS

- **Major complication of PNH**
  - Refractory, transfusion-dependent hemolytic anemia
  - Recurrent, life-threatening thromboembolic disease
  - Patient Circumstances, Including Preference?

HSCT for PNH

- There are no PNH-specific adverse events.
- Severe, acute graft vs. host disease occurs in approximately 33% of patients and the incidence of chronic graft vs. host disease is roughly 35%
- Overall survival for unselected PNH patients who undergo transplantation using an HLA-matched sibling donor is in the range of 50% to 60%
News from ASH 2010

• 79 patients treated with eculizumab from 2002-2010
  – Median age at dx, 37; at treatment, 46
  – Median clone size, 96%
  – No difference in survival compared to age and sex matched controls.
  – Three deaths were from non-PNH causes
  – 1 AML, 2 MDS
  – 1 spontaneous remission

Kaplan-Meier survival plots depicting PNH patients on eculizumab compared to age and sex matched controls

The End

• Thank you