PNH—An Overview

Charles J. Parker, M. D.

Division of Hematology and Hematological Malignancies
University of Utah School of Medicine
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

**Normal Control**

**Patient**

**RBCs**

**PMNs**

CD55 + CD59

72%

96%
• 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 4\textsuperscript{th} 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

Normal Hematopoietic Cells

PNH Hematopoietic Cells

glycosylphosphatidylinositol (GPI)-anchored proteins

transmembrane protein

membrane lipid bilayer

UDP-GlcNAc + PI $\rightarrow$ GlcNAc-PI

mutant PIG-A

X Chromosome
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

PNH RBC09.002

0%

PNH02.002

3%

PNH18.002

0.077%

PNH WBC09.002

0.001%

PNH04.002

21%

PNH09.002

0.747%

PMNs
Flow Cytometric Diagnosis of PNH

Patient

Normal Control

RBCs

PMNs

CD55 + CD59

CD55 + CD59

72%

96%
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

- Selection Pressure
- Bone Marrow
- PIGA mutant HSCs
- Clonal expansion
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

porto  rose’  Chablis
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), haptoglobin, iron stores
• Bone marrow aspirate, biopsy, and cytogenetics
## Classification of PNH*

<table>
<thead>
<tr>
<th>Category</th>
<th>Rate of Intravascular Hemolysis†</th>
<th>Bone Marrow</th>
<th>Flow Cytometry</th>
<th>Benefit from Eculizumab</th>
</tr>
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<tbody>
<tr>
<td><strong>Classic</strong></td>
<td>Florid (macroscopic hemoglobinuria is frequent or persistent)</td>
<td>Cellular marrow with erythroid hyperplasia and normal or near-normal morphology††</td>
<td>Large population (&gt;50%) of GPI-AP deficient PMNs¶</td>
<td>Yes</td>
</tr>
<tr>
<td>PNH in the setting of another bone marrow failure syndrome§</td>
<td>Mild to moderate (macroscopic hemoglobinuria is intermittent or absent)</td>
<td>Evidence of a concomitant bone marrow failure syndrome§</td>
<td>Although variable, the percentage of GPI-AP deficient PMNs¶ is usually relatively small (&lt;30%)</td>
<td>Dependent on the size of the PNH clone</td>
</tr>
<tr>
<td><strong>Subclinical</strong></td>
<td>No clinical or biochemical evidence of intravascular hemolysis</td>
<td>Evidence of a concomitant bone marrow failure syndrome§</td>
<td>Small (&lt;1%) population of GPI-AP deficient PMNs detected by high-resolution flow cytometry</td>
<td>No</td>
</tr>
</tbody>
</table>

* Based on recommendations of the International PNH Interest Group (Blood 2005;106:3699-3709)
† Based on macroscopic hemoglobinuria, serum LDH concentration and reticulocyte count
†† Karyotypic abnormalities are uncommon
§ Aplastic anemia & refractory anemia/MDS are the most commonly associated marrow failure syndromes
¶ Analysis of PMNs is more informative than analysis of RBCs due to selective destruction GPI-AP deficient RBCs
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

• Intravascular 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
Management of Thrombosis

- Anticoagulation with warfarin
  - Requires monitoring
- Anticoagulation with LMWH
  - Requires SQ injection daily
- New oral anti-thrombin and anti-Xa inhibitors
  - Do not require monitoring
  - Limited on-label use
  - Expensive until competitive prices takes effect
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 selected for PIGA-mutant GPI-AP deficient HSCs.
• The basis of the selection is speculative.
• A young patient with PNH
• The photographs are from adjacent fields in the same biopsy specimen

Stephen Richards, Leeds, UK
GPI-AP Deficient Granulocytes in Bone Marrow Failure Syndromes

- **AA**
  - N=303

- **AA-PNH**
  - N=8

- **PNH**
  - N=9

- **RA**
  - N=170

- **RAEB**
  - N=61

- **control**
  - N=104

- **56.4%** (171/303)
- **19.4%** (33/170)
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 a 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

C3a

C5 convertase
C3b Bb C3b P

C5a

Membrane Attack Complex
C5b-9n

*CD55-

*CD59-

*GPI-anchored complement regulatory proteins deficient in PNH

Complement Activation

Normal RBC

PNH RBC
**Generation of C3 Opsonins* on Erythrocytes**

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

- CR2 → C3dg
- CR3 → iC3b
Eculizumab Treatment Alters the Natural History of PNH

A

H17/3E7

H2O\cdot C3Bb

C3bBbP

C3 convertase

C3 convertase

C3a

C5 convertase

C5 convertase

C5a

C5b-9n

PNH Untreated

PNH + eculizumab

PNH + H17/3E7

No hemolysis or C3 opsonization

B

hemolized RBC

C3b

iC3b

C3dg

opsonized RBC

PNH Untreated

PNH + eculizumab

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 \textit{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?
  - Potential for cure vs. life-long, chronic therapy
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