What is PNH?

- **Paroxysmal** – sudden onset
- **Nocturnal** – occurring at night (or early in morning upon awakening)
- **Hemoglobinuria**

Despite the name, most patients do not present this way.

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**PAROXYSMAL NOCTURNAL HEMOGLOBINURIA**

- Estimated 4,000 – 6,000 patients in U.S.\(^1\)
- 5 year mortality: 30%\(^2\)
- Diagnosed at all ages – Median age early 30\(^{st}\)\(^3,4\)
- Quality of life diminished\(^5\)
- Progressive disease\(^6\)

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CAUSES OF PNH

WHAT CAUSES PNH?

• The mutation in the PIG-A gene in a hematopoietic stem cell leads to a defect in the production of an anchor protein that ties other proteins to the cell surface.

STEM CELLS

- Embryonic Stem Cell
- Somatic Stem Cells
- The Cells of Each Specific Organ

- Egg or Sperm
- Blood
- Muscle
- Nerve
- Etc.
A blood stem cell can give rise to all the cells of the blood.

IN PNH, a single mutation occurs in one blood stem cell.

Evolution of PNH in marrow.
PNH: PATHOPHYSIOLOGY

Stem cells

GPI-anchored proteins

MUTATIONS IN PIG-A GENE IN PNH

Somatic mutations in PIG-A gene

Lack of GPI linked proteins is responsible for symptoms:
- Hemolysis
- Thrombosis
- Escape from immune attack and selection

Lack of surface GPI Anchored Proteins

GPI-LINKED PROTEINS

Examples:
- Decay accelerating factor CD59
- Membrane inhibitor or reactive lysis CD58
- LPS receptor CD14
- LFA-3 CD59
- CD48
- Leukocyte alkaline phosphatase
- Acetylcholine esterase
- Thy-1
- Urokinase plasma receptor, UPAR
- CD177 (NA-1)
- And many more……
IMMUNE THEORY OF PNH EVOLUTION

[Diagram showing immune attack on hematopoietic cells, normal stem cell, PNH stem cell, T-cells, selective pressure, and selective resistance.]

WHAT IS COMPLEMENT?

- Complement is a group of proteins that are part of our immune system.
- Complement circulates in an inactive form.
- A little bit of complement is always being activated spontaneously, especially at night.
- Many different events can activate complement including trauma, infection, stress, etc.
- Complement will attack certain bacteria by making pores in the surface of the bacteria.
- In PNH, activated complement will attack red cells causing them to “lyse” (burst)

Terminal Complement Activation Renders RBCs Susceptible to Hemolysis

[Diagram showing normal RBCs, CD59, intact RBC, PNH RBC, complement activation, chronic hemolysis, lysed PNH RBCs, and free hemoglobin in the plasma.]
WHAT HAPPENS WHEN RED CELLS HEMALYZE?

- The red cells are destroyed - anemia
- Hemoglobin is released into the plasma (the fluid part of blood)
- Some of the hemoglobin passes through the kidneys and into the urine leading to the dark color of the urine
  - Loss of iron
  - May lead to kidney damage in the long run
- Free hemoglobin binds nitric oxide
  - What is nitric oxide?

WHAT IS NITRIC ACID?

- A gas produced by the body to regulate smooth muscle cells.
- An increase in free nitric oxide causes smooth muscle cells to relax. A decrease causes smooth cells to contract.
- Smooth muscle cells are in many tissue
  - Blood vessel walls: ischemia, impotence
  - Esophagus and GI tract: esophageal spasm, reflux, abdominal pain

SYMPTOMS OF PNH
CARDINAL SYMPTOMS

- Hemolysis
- Propensity for blood clots (thrombosis)
- Decreased production of blood cells

65% of patients lived longer than 10 years and 48% longer than 15 years

Predictors of decreased survival:
- Development of low counts (relative risk of 5.5)
- Occurrence of thrombosis (relative risk 10)
- Development of MDS (relative risk of 19)

In 50% of patients PNH does not affect their survival

SIGNS AND SYMPTOMS OF PNH

<table>
<thead>
<tr>
<th>Clinical Signs or Symptoms</th>
<th>Incidence Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombosis</td>
<td>40%</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>66%</td>
</tr>
<tr>
<td>Chronic Renal Disease stage 1 – 5</td>
<td>65%</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>57%</td>
</tr>
<tr>
<td>Anemia</td>
<td>Up to 100%</td>
</tr>
<tr>
<td>Fatigue, impaired QOL</td>
<td>96%</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>26%</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>41%</td>
</tr>
<tr>
<td>Erectile Dysfunction</td>
<td>47%</td>
</tr>
</tbody>
</table>

PNH - Thrombosis

- 10% of PNH patients present with thrombosis (blood clots)
- Up to 40% will develop clots during the course of their disease.
- While approximately 1/3 of these clots are typical deep vein thrombosis, another 1/3 occur in the GI system (liver, spleen), and the last third are in unusual locations (CNS, dermal veins, arterial sites).
- Once a clot develops, there is a strong tendency towards developing further clots, even with anticoagulation.
- Once a thrombosis develops, the prognosis is grim.
DIAGNOSING PNH
**DIAGNOSIS OF PNH**

- Clinical symptoms/suspicion
  - History of aplastic anemia
  - Fatigue – anemia
  - Hemolysis
  - Sudden blood clots
  - Dark urine

- Laboratory testing
  - Low blood counts
  - Hemolysis
    - LDH high
    - Haptoglobin low
    - Often low iron
    - Flow cytometry for PNH cells
    - Often high reticulocytes
    - Bone marrow exam

**CAUSES OF HEMOLYTIC ANEMIA**

**EXTRINSIC CAUSES**
- Immune mediated
  - AIHA
  - Drug induced
  - Delayed transfusion rx
  - PCH
  - Cold agglutinin dz
- Microangiopathic
  - DIC
  - TTP/HUS
  - Valve hemolysis
  - Other trauma (March, burns)
- Other causes
  - Snake bites
  - Toxins
  - Infusion of hypotonic sol’n

**INTRINSIC CAUSES**
- Enzyme deficiencies
  - G6PD
  - Pyruvate kinase
  - Others
- Hemoglobinopathies
  - Sickle cell dz
  - Thalassemia
  - Unstable Hgb
- Membrane defects
  - Hereditary spherocytosis
  - Hereditary elliptocytosis
- Paroxysmal Nocturnal Hemo
globinuria (PNH)

**METHODS OF HISTORICAL INTEREST**
- Ham Test – acidified serum lysis test
  - Specific but not sensitive
- Sugar Water Test – serum in isotonic sucrose solution
  - Sensitive but not specific
- Complement lysis sensitivity test – lysis by antibody and limiting complement
  - Defined PNH II (moderately abnormal)
  - and PNH III (markedly abnormal red blood cells)
Flow Cytometry: Diagnostic Test for PNH

- Perform on peripheral blood
- Use monoclonal antibodies against GPI-anchored proteins, such as CD59 or CD55\(^1,2\)
- PNH blood cells (PNH clone) are cells missing GPI-anchored proteins


Fluorescent AERolysin (FLAER)

- FLAER binds to the GPI-anchor itself, rather than to a single protein such as CD55 or CD59
- FLAER provides much greater signal noise and better accuracy than an antibody against a single target

Types of PNH
TYPES OF PNH

Primary hemolytic PNH
- No evidence for marrow failure
- Can evolve from AA
- Often primary disease
- Anemia solely due to hemolysis
- No benefit of immunosuppression

AA/PNH syndrome
- Counts often depressed
- Marrow hypocellular
- May benefit from immunosuppression
- Usually a late complication of AA

CLINICAL MODES OF DEVELOPMENT OF PNH

Primary hemolytic PNH
- Hemolysis

Aplastic anemia
- Secondary hemolytic PNH
- Aplastic anemia/PNH syndrome
- Bone marrow failure

PNH CLONES IN BONE IMMUNE MEDIATED BONE MARROW FAILURE

% of GPI-deficient granulocytes

CONTROL   AA      MDS     PNH