PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Rare but Real

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Discuss symptoms and complications of PNH

Discuss pathophysiology of PNH

Identify clinical categories of PNH

Identify high risk patients

Discuss laboratory diagnosis for PNH

Overview of management of PNH
Paroxysmal Nocturnal Hemoglobinuria (PNH)

- Rare benign clonal acquired hematopoietic stem-cell (HSC) disorder
- Somatic mutation of X-linked phosphatidylinositol glycan class A (PIGA) gene
- Can arise *de novo* or in the setting of acquired bone marrow (BM) failure
- Product of PIGA gene is required for synthesis of anchor protein that ties other proteins to the cell surface known as glycosylphosphatidylinositol (GPI-anchor)
- Two GPI-anchored proteins (CD55 & CD59) normally function as complement regulatory proteins

**CD59**
- Membrane inhibitor of reactive lysis (MIRL)
- Forms defensive shield for RBCs
- Inhibits the assembly of the membrane attack complex

**CD55**
- Decay accelerating factor (DAF)
- Prevents formation and augments instability of C3 convertase
Alternative Pathway of Complement

C3 convertase
C3bBbP
CD55*

C5 convertase
C3bBbC3bP
CD55*

Membrane Attack Complex
C5b-9n
CD59*

*CPI-anchored complement regulatory proteins deficient in PNH
Paroxysmal Nocturnal Hemoglobinuria (PNH)

- Deficiency can be partial or complete
- Seen in WBCs and RBCs
- Characterized by continuous destruction of PNH RBCs due to vulnerability to complement mediated lysis
RBCs Susceptible to Lysis by Terminal Complement Activation

- Normal RBC
- Intact RBC
- PNH RBC
- Chronic hemolysis & free hemoglobin in plasma

CD59

Complement Activation
Paroxysmal
   Destructive progressive ongoing hemolysis even in the absence of symptoms

Nocturnal
   Hemolysis in PNH is subtle and constant  24 hours a day

Hemoglobinuria
   Is a less commonly seen complication ~ 75% of patients present without hemoglobinuria
Clinical Manifestations of PNH

- Fatigue, impaired quality of life
- Anemia
- Dyspnea
- Chronic kidney disease
- Abdominal pain
- Pulmonary hypertension
- Erectile dysfunction
- Dysphagia
- Thrombosis
- Hemoglobinuria
- Bone marrow failure

PNH symptom incidence rate:

- 41% Dysphagia
- 50% Pulmonary hypertension
- 66% Dyspnea
- 57% Abdominal pain
- 64% Chronic kidney disease (CKD)
- 47% Erectile dysfunction
- 26% Hemoglobinuria
- 40% Thrombosis
- 88% Anemia
- 96% Fatigue, impaired QoL

Alexion Pharmaceuticals
PNH easy to miss. Impossible to ignore

Impaired QoL regardless of clone size

<table>
<thead>
<tr>
<th>Condition</th>
<th>Gran clone &lt;10%</th>
<th>Gran clone 10%-49%</th>
<th>Gran clone &gt;50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>59</td>
<td>72</td>
<td>76</td>
</tr>
<tr>
<td>Discolored urine</td>
<td>30</td>
<td>56</td>
<td>49</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>44</td>
<td>44</td>
<td>53</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>41</td>
<td>53</td>
<td>46</td>
</tr>
<tr>
<td>Chest pain</td>
<td>14</td>
<td>31</td>
<td>24</td>
</tr>
</tbody>
</table>

N=580
what you can’t see can hurt you the most..
Pathophysiology of PNH

• **Hemolysis**
  ◦ Complement activation

• **Chronic kidney disease**
  ◦ Toxicity of free hemoglobin and iron with extensive hemoglobin deposition

• **Esophageal spasm, abdominal pain, pulmonary hypertension, fatigue and smooth muscle dystonia**
  ◦ Nitric oxide scavenging

• **Thrombosis**
  ◦ Nitric oxide depletion
  ◦ Disrupted fibrinolysis
  ◦ Disrupted tissue factor inhibitor pathway

• **Bone marrow failure**
  ◦ PNH is closely related to bone marrow failure syndromes
  ◦ Mutant HSC exhibit a survival advantage and tend to expand leading to hemolysis
Pathophysiology of PNH

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**Clinical Categories of PNH**

1. **Classical PNH, includes hemolytic and thrombotic patients**
   - Marked hemolysis
   - Hemoglobinuria
   - Lactate dehydrogenase (LDH)
   - Normal BM with erythroid hyperplasia
   - PNH clone >50%

2. **PNH in the setting of BM failure syndromes**
   - Mild hemolysis
   - Minimal abnormality in biochemical markers of hemolysis
   - BM shows the concomitant BM failure
   - PNH clone usually small (<10%)

3. **Subclinical PNH**
   - No clinical or biochemical evidence of intravascular hemolysis
   - BM shows concomitant BM failure
   - Small PNH clone (<1%)
Early Diagnosis is Essential for Improved Patient Management and Prognosis

International Clinical Cytometry Society (ICCS) Guidelines and International PNH Interest Group (IPIG) recommend evaluation of high risk patients:

- Coombs negative hemolytic anemia (22.7%)
- Hemoglobinuria (18.9%)
- Aplastic anemia (AA) (26.3%)
- Refractory anemia-myelodysplastic syndrome (RA-MDS) (17%)
- Unexplained venous or arterial thrombosis (1.4%)
- Unexplained cytopenia (5.7%)
PNH Clone in Patients with AA

• Aplastic anemia: disease where the BM stops making RBCs, WBCs, and platelets

• PNH clone present in 40-50% of patients with severe AA

• PNH clone size in patients with AA may increase rapidly and unpredictably

• Presence of PNH clone in severe AA is associated with low morbidity and mortality, and reported to be predictive of response to immunosuppressive therapy

• Clone size often decreases after immunosuppressive therapy
PNH Clone in Patients with MDS

- Myelodysplastic syndrome: group of diverse BM disorders where BM does not produce enough healthy blood cells

- More than 1 out of 18 patients with MDS have PNH clone

- Most studies showed that PNH clones were only present in patients with RA-MDS

- PNH clone in other categories of MDS has been reported in limited number of studies

- RA-MDS patients with detectable PNH clone have more indolent clinical course
Diagnosis of PNH

- Sucrose hemolysis test and Ham test, both show increased sensitivity of PNH RBCs to complement-mediated hemolysis under standard conditions (lows specificity and sensitivity, miss small populations)

- Complement lysis sensitivity, measures the amount of hemolysis at different concentrations of complement (laborious, difficult to standardize, miss small populations)

- Mutation analysis can provide final confirmation, however mutations are common in normal individuals which are usually polyclonal and occur in differentiated progenitors
Diagnosis of PNH

• Flow-cytometry performed on peripheral blood is the established method of choice for the diagnosis and monitoring of PNH

• Both RBCs and WBCs should be tested
  ◦ WBC PNH clone can sometimes be detected in the absence of a RBC clone
  ◦ A significant RBC PNH clone is always associated with a WBC clone
  ◦ Explained by the fact that RBC clone size may be affected by hemolysis or transfusion
RBC Analysis

- Identify and quantify cells lacking expression of CD59 or CD55 (Type III)

- Identify and quantify cells partially deficient (Type II) if present

- Glycophorin-A (CD235a): lineage marker used to gate on RBCs

- CD59 is superior over CD55
A

B

C

D

RBC Analysis

RBC Analysis

Type I = 95.2
Type II = 4.72
Type III = 0.02%

Sutherland DR et al. Cytometry Part B 2012;82B:195–208
RBC Analysis

Sutherland DR et al. Cytometry Part B 2012;82B:195-208
RBC Analysis

A

Glycophorin FITC-A

0% 0% 100%

Type III Type II Type I

B

Glycophorin FITC-A

41% 41%

Type III Type I

C

Glycophorin FITC-A

19% 13% 19% 13%

Type III Type II Type I

ARUP LABORATORIES
High Sensitivity RBC Analysis

- Count more RBCs for small PNH clone
- Sensitivity of 0.005 % for RBCs is achievable
Granulocytes
- Most commonly used to assess PNH clone size
- Occasionally Type II granulocytes can be detected

Monocytes
- Analyzed to confirm the granulocyte PNH clone
- Monocytes clone size often higher than granulocyte clone
- Sensitivity and precision is lower due to lower cell number
- Occasionally Type II can be detected
WBC Analysis

• **Lineage specific gating for higher sensitivity**
  ◦ CD15 to gate on granulocytes
  ◦ CD64 or CD33 to gate on monocytes

• **Assess two GPI linked proteins on each cell population**
  ◦ CD24 or CD157 and FLAER are evaluated on granulocytes
  ◦ CD14 or CD157 and FLAER are evaluated on monocytes

• **FLAER (fluorescein-labeled pro-aerolysin) is a fluorechrome conjugated inactive bacterially derived channel forming protein; binds specifically to GPI anchors**
High Sensitivity WBC Analysis

• Useful for the diagnosis of subclinical PNH associated with BM failure disorders

• Not needed for the diagnosis of classic PNH

• Sensitivity of 0.01% for WBC is achievable

• Acquisition of sufficient events, evaluation of multiple parameters and assessing frequency of PNH cells in normal samples are critical to limit false positives
WBC Analysis

A
Monocytes
CD157 PEA
97.7%
CD64+/CD157+/FLAER+

FLAER FITC-A

B
Monocytes
CD157 PEA
0.34%
PNH MONO

CD157+/FLAER+

FLAER FITC-A

A
Granulocytes
CD157 PEA
96.9%
CD15+/CD157+/FLAER+

Granulocytes
CD15+/CD157+/FLAER+

FLAER FITC-A

B
Granulocytes
CD157 PEA
0.25%
PNH PMN

CD15+/CD157+/FLAER+

FLAER FITC-A
WBC Analysis

![WBC Analysis Diagram]

Type III PNH 54.8%
Type II PNH 6.1%
WBC/RBC Analysis
Treatment of PNH

- Folic acid supplementation and supportive care for patients with minimal symptoms
- Blood transfusion
- Steroids
- Prophylactic anticoagulation: not been proven to decrease risk of thrombosis
- Allogenic BM transplantation
- Eculizumab (Soliris)
Treatment of PNH

Eculizumab

• Humanized monoclonal antibody that effectively blocks complement activation at C5 that inhibits terminal complement activation
• Stops hemolysis and related effects
• Markedly reduces transfusion requirements
• Patients with subclinical clones are not candidates for treatment with eculizumab
• Blocking the terminal portion of complement predisposes to Neisseria
• Expensive
• Must be given IV every 12-14 days
Alternative Pathway of Complement

C3 convertase
C3bBbP
CD55*

C3

C5 convertase
C3bBbC3bP
CD55*

C5

Membrane Attack Complex
C5b-9n
CD59*

*GPI-anchored complement regulatory proteins deficient in PNH

eculizumab

Charles J. Parker Hematology 2011;2011:21-29
Treatment of PNH

Survival of PNH patients receiving best historical care

35% of PNH patients die within 5 years of diagnosis despite best historical care.
Treatment of PNH

Overall Survival of Patients Before and After Eculizumab

A. Matched time periods per patient prior to and during eculizumab therapy

Thrombosis Event Rate (TE per 100 pt-years)

<table>
<thead>
<tr>
<th>Event Rate</th>
<th>Pre-eculizumab Therapy</th>
<th>During eculizumab Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events</td>
<td>39</td>
<td>3</td>
</tr>
<tr>
<td>n</td>
<td>195</td>
<td>195</td>
</tr>
</tbody>
</table>

P = .0001

Hillmen P Hematology 2008;2008:116-123
RBC PNH Clone Size in a Patient Treated with Eculizumab

A

29%

TYPE III

CD 59 PE-A

B

97%

TYPE III

TYPE I

CD 59 PE-A

C

97%

CD157

FLAER FITC-A

CD15+/CD157+/FLAER+

PNH PKN
Follow-up Testing

• Patients with established diagnosis of PNH should monitor the size of the PNH clone at regular intervals (if disease is stable, annual monitoring is sufficient)

• Any changes in clinical or hematologic parameter requires more frequent monitoring whether these show worsening or improvement

• Small clones in AA should be monitored because of the risk of developing hemolytic PNH

• Monitoring of RBC PNH clone is useful for assessing response to eculizumab therapy

<table>
<thead>
<tr>
<th>Clone size</th>
<th>&lt;0.1%</th>
<th>0.11-1.0%</th>
<th>1.01-10%</th>
<th>10.01-100%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended Follow up</strong></td>
<td>6-12 months</td>
<td>3-6 months</td>
<td>3-6 months</td>
<td>As indicated</td>
</tr>
</tbody>
</table>
Management of PNH Based on Disease Classification

Classify PNH based on flow cytometric characteristics, reticulocyte count, serum LDH concentration, bone marrow analysis

- Subclinical PNH
  - No specific PNH therapy—focus on underlying BMF syndrome

- PNH/BMF syndrome
  - Focus on BMF
    - Patients with large PNH clones may benefit from eculizumab

- Classic PNH
  - Treat with eculizumab
    - Inadequate response
      - BMT, [steroids, splenectomy], supportive care

BMF, bone marrow failure (aplastic anemia and low risk MDS); BMT, bone marrow transplant
*Some, but not all, studies suggest a favorable response to immunosuppressive therapy (IST)
†BMT eradicates the PNH clone, and typically, treatment with IST does not affect PNH clone size
‡<10% of patients with PNH/BMF have PNH clone size >50%
§Some patients respond to Danazol as first line therapy
**Consider for patients with clinically significant extravascular hemolysis
Conclusion

- PNH is a clonal hematopoietic stem cell disease
- Mutation in PIG-A gene resulting in complement mediated hemolysis
- Clinically presents with: anemia, thrombosis, cytopenia, renal failure, pains, and impaired quality of life
- Diagnosis: Flow Cytometry for RBCs and WBCs (GPI-anchored proteins)
- Treatment: Eculizumab (Ab C5)
References


