Current Thinking on the Diagnosis and Management of PNH

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What Is PNH?
(more than a hemolytic anemia)

- A disorder of the hematopoietic stem cell
- 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 insufficiency or failure

Table 1. Glycosyl Phosphatidylinositol-Anchored Proteins Deficient in PNH

| Complement Regulatory Proteins† | CD55 (decay accelerating factor, DAF) | CD59 (membrane inhibitor of reactive lysis, MIRL) |
| Complement proteins with immunological significance | | |
| Fc receptors | CD16b (Fc receptor gamma IIIb, FcRγIIIb) |
| Lipid membrane receptor | CD87 (urokinase plasminogen activator receptor, uPAR) |
| Folate receptor |
| Cellular prion protein (on resting platelets) |
| | CD24 |
| | CD48 |
| | CD52 |
| | CD66c |
| | CD66b |
| | CD90 |
| | CD108 |
| | p50-80, GP109, GP157, GP175, GP500 |

† Deficiency of complement regulatory proteins underlies the hemolytic anemia of PNH
PNH

- Pathophysiology
- Diagnosis
- Management

Characteristics of PNH

- PNH is not a binary disease as the peripheral blood of patients is a mosaic of normal and abnormal cells
- The percentage of PNH cells varies greatly among patients
- PNH erythrocytes are either completely or partially deficient in GPI-AP's
  - Type III cells are completely deficient
  - Type II cells are partially deficient
  - Type I cells are residual normal cells

Table 2: Signs and Symptoms of PNH

<table>
<thead>
<tr>
<th>Subjective</th>
<th>Objective</th>
<th>Laboratory Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue, lethargy, other loss of sense of well-being</td>
<td>Hemoglobinuria*</td>
<td>Cytopenias (anemia, thrombocytopenia, leukopenia, pancytopenia)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>Scleral icterus, jaundice</td>
<td>Evidence of intravascular hemolysis (elevated LDH, elevated indirect bilirubin, low haptoglobin)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>Thromboembolic events†</td>
<td>Flow cytometry demonstrates deficiency of GPI-AP on a portion of red cells, granulocytes and monocytes (lymphocytes are also usually involved but less so than myeloid cells)</td>
</tr>
<tr>
<td>Chest pain</td>
<td>Male impotence</td>
<td>Hemoglobinuria</td>
</tr>
<tr>
<td>Odynophagia</td>
<td>Headache</td>
<td>Iron deficiency</td>
</tr>
<tr>
<td>Male impotence</td>
<td>Headache</td>
<td>Hemosiderinuria</td>
</tr>
</tbody>
</table>

* A presenting symptom in approximately 25% of cases
† May involve an unusual site (skin, splanchnic veins [Budd-Chiari syndrome], cerebral veins)

Depends on PIGA genotype
Phenotypic Mosaicism Based on Flow Cytometry

Endo et al. Blood 1996;87:2546-2557

Patients with a high proportion of PNH II cells have disease characterized by brisk intravascular hemolysis.

Patients with a high proportion of PNH III cells have low-level chronic hemolysis.

Patients with a small proportion of PNH cells have little chronic hemolysis.

PNH

- Pathophysiology
- Diagnosis
- Management

Table 3. Basic Studies for Evaluation and Management of PNH
- Complete blood count
- Reticulocyte count
- Serum concentration of lactate dehydrogenase (LDH), bilirubin (Fractionated), haptoglobin
- Flow cytometric analysis of erythrocytes and granulocytes for expression of GPI-Aps
- Bone marrow aspirate & biopsy and cytogenetic analysis
- Serum erythropoietin concentration
- Serum concentration of blood urea nitrogen (BUN) and creatinine
- Serum iron studies (iron concentration, total iron binding capacity, transferrin saturation, ferritin concentration)
- Urine hemosiderin*

* Urine hemosiderin is indicative of chronic hemolysis but provides no quantitative information
Table 4. Classification of PNH*

<table>
<thead>
<tr>
<th>Category</th>
<th>Rate of Intravascular Hemolysis†</th>
<th>Bone Marrow Flow Cytometry</th>
<th>Benefit from Eculizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Florid (markedly abnormal LDH often with episodic macroscopic hemoglobinuria)</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Bone marrow due to erythroid hyperplasia and normal or near-normal morphology††</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>PNH in the setting of another bone marrow failure syndrome §</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>PNH in the setting of another BMF syndrome</td>
<td></td>
<td></td>
<td>Yes</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 or low risk myelodysplastic syndrome
¶ Analysis of PMNs is more informative than analysis of RBCs due to selective destruction GPI-AP deficient RBCs

**Classification of PNH Guides Management**

- **Subclinical PNH**
  - A product of screening peripheral blood from patients with BMF using high-sensitivity flow cytometry

**Basic Evaluation for PNH**

- Flow cytometric evidence of a population of peripheral blood erythrocytes and granulocytes partially or completely deficient in multiple glycosyl phosphatidylinositol-anchored proteins (GPI-APs)*
- Complete blood count; reticulocyte count; biochemical markers of hemolysis [serum concentration of lactate dehydrogenase (LDH), bilirubin (fractionated) and haptoglobin]; determination of iron stores
- Bone marrow aspirate, biopsy, and cytogenetics§

* PNH clone size is determined by the percentage of GPI-AP deficient PMNs
† The most important surrogate marker for intravascular hemolysis
§ Bone marrow aspirate and biopsy are used to distinguish classic PNH from PNH in the setting of another bone marrow failure syndrome. Nonrandom karyotypic abnormalities are rare in PNH

**Fate of PNH-type cells**

- **Phase 1**
  - Expansion: Median 15%
  - Persistence: 60%
  - Disappearance: 25%

- **Phase 2**

**Response to IST and failure-free survival in patients with aplastic anemia**

- Cumulative response rate to ATG+CsA therapy
- Failure-free survival

**PNH-AA, PNH/MDS**

- **Subclinical PNH**
  - The association between PNH and aplastic anemia has been appreciated for nearly 60 years and was refined using high-sensitivity flow cytometry.
  - The association between PNH and low risk MDS was characterized using high-sensitivity flow cytometry.

**PNH-type cells are detectable only in patients with AA and MDS-RA or RCMD**


**Fate of PNH-type cells**


- Cumulative incidence of PR P<0.01
- Probability of Failure-free survival

PNH/Aplastic Anemia and Low risk MDS

- The presence of a PNH clone appears to be a surrogate marker for immune pathology in patients with aplastic anemia and low risk MDS
- Finding of a PNH clone in the setting of aplastic anemia and MDS predicts a favorable response to immunosuppressive therapy

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 bone marrow failure syndrome*

- PNH/BMF syndrome
  - Focus on bone marrow failure†
  - Patients with large PNH clones may benefit from eculizumab¶

- Classic PNH
  - Treat with eculizumab§

*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

BMF, bone marrow failure (aplastic anemia and low risk MDS); BMT, bone marrow transplant

Suboptimal Response to Eculizumab

- A small minority of patients with classic PNH experience only modest improvement in constitutional symptoms
- Although serum LDH concentration returns to normal or near normal in all PNH patients treated with eculizumab, anemia and reticulocytosis persists in most patients with classic PNH and some remain transfusion dependent
Alternative Pathway of Complement Activation on Erythrocytes

C3 convertase
C3bBbP

C5 convertase
C3bBbC3bP

Membrane Attack Complex
C5b-9

C3a
C5a

CD59*

Complement Activation

Normal RBC
PNH RBC

Generation of C3 Opsonins* on Erythrocytes

C5 Convertase
C3 opsonins

C3 Opsonins

* C3 opsonins, iC3b and C3dg, target RBCs for destruction by reticuloendothelial cells expressing complement receptors: CR1, CR2, CR3, CR4

Management of PNH Based on Disease Classification

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

Subclinical PNH
PNH/BMF syndrome
Classic PNH

No specific PNH therapy—focus on underlying BMF syndrome*
Focus on BMF† Patients with large PNH clones may benefit from eculizumab
Treat with eculizumab
Increase dose/frequency of eculizumab, 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
** Carefully consider for patients with clinically significant extravascular hemolysis
†† Transfusion

Allogeneic SCT for PNH

• The PNH clone can be eradicated by allogeneic hematopoietic stem cell transplant
• In the era of complement inhibitory therapy, is there an indication for allogeneic BMT?

Indications for Transplant Before 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

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?
Allogeneic SCT for PNH

- Transplant related mortality is in the range of 20%-35%
- Chronic GvHD ranges from 25-75% depending on transplant regimen
- However, patients who survive transplant usually have a good to excellent performance status
- Myeloablative and non-myeloablative regimens are effective in eradicating the PNH clone

Survival of Patients with PNH Treated with Eculizumab

Kelly et al. Blood 2011 117 6786-6792

Abs, just the person I was looking for.