Paroxysmal Nocturnal Hemoglobinuria: Understanding the Diagnosis, Complications and Treatment Options

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April 21, 2018

Paroxysmal Nocturnal Hemoglobinuria

- PNH was first reported in the medical literature in the latter half of the 19th century.
- It was so named because of the mistaken belief that hemolysis (red blood cell break down) and subsequent hemoglobinuria (free hemoglobin in urine) occurred:
  - Intermittent episodes (paroxysmal)
  - Greater frequency during the night (nocturnal).

Epidemiology

- The prevalence is estimated to be between 0.5-1.5 per million people in the general population.
- PNH is believed to affect males and females in equal numbers, although some studies show a slight female preponderance.
- The disorder has been described in many ethnic groups and has been identified in all areas of the world.
  - It may occur with greater frequency in individuals of Southeast Asia or the Far East who experience greater rates of aplastic anemia.
- It can affect any age group.
  - The median age at diagnosis is during the 30s.

PNH Stem Cell

- PNH originates from a defect in a multipotent hematopoietic stem cell.
- It can arise de novo or in the setting of an underlying bone marrow failure disorder such as:
  - Aplastic Anemia
  - Myelodysplasia
  - Primary Myelofibrosis

Disease Mechanism

- The disease begins with the expansion of the hematopoietic stem cell that has severe deficiency or absence of GPI—a glycolipid moiety that anchors >150 different proteins.
- GPI deficiency is the result of a somatic mutation in PIG-A gene
  - Phosphatidylinositol glycan anchor biosynthesis, class A

- PIG-A gene is found in the X chromosome
  - A single hit is enough to generate the PNH phenotype.
  - Males only have one X chromosome
  - Females undergo lyonization (chromosome inactivation).
- The mechanism for the mutations is unknown.
  - Typical mutagenic events such as radiation, chemotherapy, etc. rarely exist.
  - To cause PNH, the stem cell needs to undergo clonal selection and expansion.
• PIG-A gene is responsible for the first step in the synthesis of GPI anchor that attaches a subset of proteins to the cell surface.

• Leads to a deficiency in complement inhibitory proteins: CD55 and CD59 which leads to chronic complement mediated hemolysis of the GPI deficient red blood cells.

Classification of PNH

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<td>No hemolysis</td>
<td>Normal morphological and karyotypic abnormalities</td>
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Clinical Manifestations: Signs and Symptoms

Classical PNH

• Characterized by signs and symptoms of hemolysis or red blood cell breakdown.
• Symptoms directly attributed to anemia or the destruction of red blood cells:
  - Shortness of breath
  - Fatigue
• Symptoms indirectly associated to the release of free hemoglobin:
  - Dyspnea
  - Abdominal pain
  - Erectile dysfunction
  - Pulmonary hypertension
  - Renal dysfunction
• Hypercoagulability or tendency to clot

Fatigue - 80%
Shortness of breath - 64%
Hemoglobinuria (Free hemoglobin in urine) - 62%
Abdominal pain - 44%
Bone marrow suppression – 44%
Erectile dysfunction - 38%
Chest pain - 33%
Thrombosis – 16%
Renal Failure - 14%

Other complications:
- Iron deficiency anemia
- Headaches
- Confusion
Laboratory Abnormalities

- Anemia (low red blood cell counts)
- Note: possible to have hemolysis without anemia if the bone marrow is able to compensate.
- Increased reticulocyte count
- Increased lactate dehydrogenase (LDH) and indirect bilirubin
- Decreased haptoglobin
- Free serum hemoglobin with pink/red serum
- Hemoglobinuria with pink/red urine, positive dipstick for heme and negative sediment for red blood cells
- Negative direct antiglobulin (Coombs or DAT)

Additional Laboratory Abnormalities

- Hypocellular, normocellular or hypercellular bone marrow often with erythroid hyperplasia
- May see erythroid dysplasia
- Iron deficiency:
  - Low serum iron, low ferritin, increased transferrin, absent bone marrow iron
- Creatinine and blood urea nitrogen may be increased (BUN) from acute and/or chronic renal damage
- Liver function tests may be abnormal due to hepatic or portal vein thrombosis
- Mild reduction in blood counts may occur due to splenomegaly caused by portal vein thrombosis or splenic vein thrombosis

Thrombosis

- It leads to severe morbidity and it is the most common cause of mortality in PNH.
- Venous thrombosis is more common than arterial thrombosis.
- It may occur in any PNH patient but those with large percentage of PNH cells (>50% WBC) are at greatest risk.
- 10 year risk of thrombosis for those with >50% clone: 44% compared to 5.8% on those patients with <50%.

Thrombosis in PNH: Mechanism

- It is poorly understood.
- Platelet activation secondary to:
  - The absence of complement regulatory proteins in platelets leads to prothrombotic microparticles.
  - Free hemoglobin leads to scavenging of NO.
- Complement activation leads to increased inflammatory proteins: IL6, IL8, tumor necrosis factor α (TNF-α).
- Defective fibrinolysis resulting from deficiency or absence of GPI linked proteins such as:
  - Urokinase-type plasminogen activator
  - Heparan sulfate
  - Tissue factor pathway inhibitor
Thrombosis in PNH: Evaluation

• No evidence to support routine imaging or laboratory testing.
• Clinical suspicion!
• Rely on history and physical examination
• Baseline d-dimer may be useful to direct history taking to elicit symptoms of thrombosis
• Neurologic symptoms, abdominal pain, leg swelling
• Increased abdominal girth, large spleen and liver, neurologic deficits on exam

Who should get screened for PNH?

• Patients with hemoglobinuria.
• Patients with Coombs negative intravascular hemolysis (especially with concurrent iron deficiency)
• Patients with venous thrombosis at unusual sites:
  • Budd-Chiari (hepatic vein)
  • Mesenteric or portal veins
  • Cerebral veins
  • Dermal veins
• Patients with aplastic anemia diagnosis
• Patients with refractory anemia MDS
• Patients with episodic dysphagia (difficulty swallowing) or abdominal pain with evidence of intravascular hemolysis

How do you diagnose PNH?

• Flow cytometry analysis using antibodies directed against GPI-associated proteins:
  • CD55, CD59
  • FLAER (fluorescent labeled aerolysin):
    • Takes advantage of binding of a bacterial protein: aerolysin to GPI anchor.
    • Useful for the analysis of white blood cells but not red blood cells
  • Other information to get from flow:
    • Discrete populations with different degrees of deficiency
    • Percentage of cells that are abnormal
  • Ham and sucrose tests
    • Largely abandoned as diagnostic assays

Types of PNH cells

• Red blood cells are defined by the abundance of GPI anchored proteins on the surface:
  • PNH Type I cells: normal levels
  • PNH Type II cells: partial absence
  • PNH Type III cells: complete absence
  • Knowing the percentage and type of deficient RBC can be helpful in management of anemia.
  • A word about transfusion: It is unlikely to obscure the diagnosis BUT it can affect the calculation of proportion of cells with normal expression of GPI-AP.
    • Best to perform flow before transfusion or during a (minimum 30 days) period of transfusion abstinence.

• It is recommended that at least two independent flow cytometry reagents be used (FLAER and antibodies against monoclonal proteins) in at least two cell lineages (i.e. RBC and WBC).
• WBC (monocytes, granulocytes) are the optimal cell type for assessing the PNH clone.
• The life span of WBC is normal
• Unaffected by RBC transfusion.

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<th>PNH types</th>
<th>Clone Size in granulocytes</th>
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<tr>
<td>Classic PNH</td>
<td>40-99%</td>
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<tr>
<td>Acquired Aplastic Anemia</td>
<td>0.1-10%</td>
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<tr>
<td>MDS</td>
<td>≤1%</td>
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<tr>
<td>Healthy Individuals</td>
<td>≤0.002</td>
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Testing of RBC to assess severity of disease

• Assessment of the RBC population can be useful to determine severity of disease.
  • In general:
    • Patients with high Type III RBC have clinically apparent hemolysis.
    • A patient may have diagnosis of PNH but if the percentage of Type III RBC is low, hemolysis may not be clinically significant.
    • If RBC have partial deficiency (type II), hemolysis may be modest even if the percentage of cells is high.

Charles Parker et al. Blood 2005;106:3699-3709
Recommendations for flow cytometric analysis in diagnosis and management of PNH

- For patients with clinical evidence of hemolysis (classic PNH and PNH/aplastic anemia)
  - At diagnosis, flow cytometry of both WBC and RBC is recommended.
  - After diagnosis, every 6 months for 2 years and yearly thereafter as long as other lab parameters are stable.

- For patients with aplastic anemia or refractory anemia-MDS without clinical evidence of hemolysis
  - At diagnosis, analysis of RBC and WBC using high sensitivity flow cytometry.
  - Every year even in the absence of clinical hemolysis (including on patients treated with immunosuppressive therapy).

How to treat?

- Treatment is merited if the patient is symptomatic from PNH.
- Patients with chronic hemolysis with disabling fatigue, frequent pain paroxysms, loss of sense of well being affecting quality of life.
- The hemolysis is affecting other organs, i.e. renal dysfunction, male impotence in male, thrombosis.
- PNH clones <10% rarely require clinical intervention.
- Watchful waiting is appropriate for asymptomatic patients or those with mild symptoms.

Treatment: Hemolytic Anemia

- It is important to remember that the anemia may be due to hemolysis, bone marrow failure, iron or vitamin deficiency.
- If the reticulocyte count is inappropriately low: check for deficiencies and replete.
- Folic acid 1-2 mg daily is recommended to all patients with ongoing hemolysis due to the increased demands of accelerated erythropoiesis.

- Hemolysis can produce fatigue out of proportion with the hemoglobin level.
- If the anemia is severe (Hgb < 7 g/dL) and the patient is symptomatic (fatigue, fatigue, etc).
  - There is a lack of evidence to support glucocorticoids, androgens and erythroid stimulating agents.
  - Stimulons can reduce hemolysis but their limited efficacy and toxicity reduce them less effective.
  - Treatment with RBC transfusions is indicated
  - If the patient is transfusion dependent, consider anti-complement therapy.

Eculizumab

- A humanized monoclonal antibody against C5 that inhibits the terminal complement pathway.
- In 2007, the US FDA approved eculizumab for use in PNH based on its efficacy in two phase 3 clinical trials:
  - Eculizumab is effective in reducing intravascular hemolysis
  - Does not stop extravascular hemolysis
  - Does not treat bone marrow failure

TRIUMPH

- Transfusion Reduction efficacy and safety clinical Investigation Using eculizumab in PNH
- Randomly assigned 87 patients with severe PNH to treatment with eculizumab or placebo for 26 weeks.
- Enrollment to patients with severe disease: transfusion dependence, >10% PNH type III cells, increased LDH
- Primary endpoints: Hemoglobin level and transfusion requirement
- Secondary endpoints: LDH
- Results:
  - Greater stabilization of hemoglobin levels: 49% vs 0%
  - Fewer transfusions: transfusion independence in 51% vs 0%
  - Improved laboratory parameters: LDH

Therapy Options

- Corticosteroids (hemolytic anemia)
- Androgens (hemolytic anemia)
- Transfusion
- Splenectomy
- Iron Replacement
- Folate
- Eculizumab
- Bone marrow transplant
SHEPHERD

- Safety in Haemolytic PNH patients treated with Eculizumab: a multicenter open label Research Design
- Prospective evaluation of 97 patients treated with eculizumab for one year (more diverse population: thrombocytopenia, less severe anemia)
- Outcomes:
  - Improved hemoglobin from 9.3 g/dL to 10.2 g/dL
  - Reduced transfusion requirements (from 8 to 0 units of PRBC/patient/annually)
  - Reduced measures of hemolysis (LDH)
  - Improved Quality of Life

Monitoring patients on eculizumab

- Most patients have symptomatic improvement hours to days after the first dose.
- Weekly labs for first four weeks: complete blood count, LDH, reticulocyte count and complete metabolic profile
  - LDH will normalize within days to weeks of initiating therapy.
  - Reticulocyte count will remain elevated (still have extravascular hemolysis)
- Monthly monitoring thereafter.
- Classic PNH patients who are transfusion dependent will have a marked decrease in transfusion needs
  - Nearly 50% achieve transfusion independence.

Eculizumab: Other toxicities and cost

- Headache in 50% of patients
- Consequence of increase in nitric oxide levels.
- Improves after the first dose or two.
- Nasopharyngitis
- Back Pain
- Nausea
- Neisseria sepsis
  - 0.5% yearly risk of Neisseria sepsis even after vaccination.
  - Revaccination against N. meningitides every 3 to 5 years
- Effective in patients with classic PNH
- Treatment with eculizumab:
  - Decreases or eliminates the need for blood transfusions
  - Improves quality of life
  - Reduces the risk of thromboses
- Two weeks before starting therapy, vaccinate against N. meningitides
- Initial administration: 600 mg weekly for the first 4 weeks then 900 mg biweekly starting on week 5.

Treatment: Smooth Muscle Dystonia

- Paroxysms from smooth muscle dystonia can be debilitating.
- Opioid analgesia may be required.
- Anti-complement therapy.
Treatment: Thrombosis in PNH

- RECALL: Patients with large percentage of PNH cells (>50% WBC) are at greatest risk.

- Anticoagulation is indicated for acute treatment of thrombotic events.
  - No firm recommendations on type of anticoagulant.
  - Thrombolytic therapy can be used if clinical criteria indicate its need.

- Patients with life threatening thrombosis in the setting of low platelet counts may need to have platelet transfusions to allow the use of anticoagulants and/or thrombolytic therapy.

- Expert opinion: thrombosis is an absolute indication for initiating treatment with eculizumab.
  - Secondary prophylaxis regardless of the size of the PNH clone

- A potential exception: a patient with a very small PNH clone (too small to cause clinically significant hemolysis) and a new DVT that was provoked (risk factors associated with it that are independent of PNH diagnosis)
  - May treat these patients as you would any other who presents with a DVT

- Do you stop anticoagulation on patients with prior history of thrombosis?
  - Controversial; however, if patient is well controlled on eculizumab, may be appropriate to discontinue after 3-6 month treatment of clot

Prophylactic Anticoagulation

- Benefit of prophylactic anticoagulation:
  - A continued issue of debate among members of the International PNH group.
  - 2003 study (Hall): prophylaxis with warfarin in patients with 50% clone improved incidence of thrombosis when compared to those who received no treatment.
  - There are not enough studies to demonstrate benefit of prophylaxis in patients with PNH
  - May be dangerous if the patient also has low platelet counts
  - Not recommended at this time.

- Expert opinion: No initiation of prophylactic anticoagulation on patients not on eculizumab.
  - Possible exceptions: pregnant patients, persistently elevated d-dimer, perioperative period.

Bone Marrow Transplantation

- BMT should not be offered as initial therapy for patients with classic PNH due to the risks of transplant related morbidity and mortality.
- Exceptions:
  - PNH patients in countries where eculizumab is not available.
  - Failure to respond to eculizumab due the heterozygous mutation c.2654G→A mutations in C5
  - Failure of eculizumab to block intravascular hemolysis due to persistent inflammation.
  - For patients meeting criteria for aplastic anemia with PNH clone, BMT is a reasonable course if the patient is young and has a suitable donor

Special populations: Children

- PNH in children is rare and evidence to guide therapy is lacking.

- We manage it very similarly to what has been described in adults:
  - If symptoms of hemolysis are severe and/or thrombosis: anti-complement therapy.
  - Active surveillance for asymptomatic patients or those with mild symptoms.
  - Transplant or immunosuppressive therapy for those patients with severe aplastic anemia with PNH-clone.

Special Populations: Pregnancy

- Patients with PNH can have a successful pregnancy BUT there is an increase in morbidity and mortality to both mom and baby.

- Management recommendations:
  - Iron and folate supplementation—pregnancy increases requirements of both which are already high in PNH
  - Transfusions: As needed for management of anemia and thrombocytopenia. Transfusion requirements may increase with pregnancy.
**Special population: Pregnancy**

- Thrombosis risk reduction: Limited data to guide management.
  - Prophylactic (low dose) or therapeutic (full dose) may be appropriate depending on thrombosis history or risk (>50% PNH clone).
  - When to start: confirmation of pregnancy or 3rd trimester?
  - Consider any contraindications
  - Continue the anticoagulation through 6 weeks of postpartum period.

- Eculizumab:
  - There is some experience now with its use in pregnancy.
  - Recommend continuing it or initiating it if the patient otherwise qualifies for it.
  - Improved maternal outcomes in women with PNH without increased fetal risks

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**Thrombosis in Non-Hemolytic Paroxysmal Nocturnal Haemoglobinuria**

Morag Griffin, Talha Munir, Louise Arnold, Nicola Copeland, Kathryn Hilling, Stephen John Richards and Anita Hill
Blood 2017 130:1175

- Interrogation of the UK Leeds database:
  - Group 1: White cell clone >30%, red cell clone <10%, LDH <2xULN
  - Group 2: White cell clone >30%, red cell clone >10% with higher proportion of type II red cells than type III red cells, LDH =2xULN
  - Group 3: White cell clone 10-30%, red cell clone <10%, LDH >2xULN
  - Patients at higher risk of thrombosis in this cohort were those with non-hemolytic PNH, high white cell clones and low red cell clones (group I).
  - Those with a greater proportion of type II red cell clones compared to type III red cells and LDH <2x ULN were at risk of thrombosis. This is an under-recognized risk for patients with PNH cells.
  - This study supports the theory that while hemolysis may be associated with a thrombotic event, white cell and platelet factors have a more pivotal role than previously thought in the mechanisms of thrombosis in PNH.

**Paroxysmal Nocturnal Hemoglobinuria Results from Initial Clonal PIG-a Mosaicism**

Michael J. Clemente, Bartlomiej Przychodzen, Cassandra M. Hirsch, Bhumi J. Patel, Talha Bat, Yasunobu Nagata, Marcin W. Wlodarski, Hideki Makishima and Jaroslaw P. Maciejewski
Blood 2017 130:1179

- The pathogenesis of PNH involves alteration of at least one HSC clone, characterized by a somatic mutation in the PIG-A gene.
- In animal models, failure of PIG-A mutant clones to expand suggests that additional factors are necessary for the development of PNH.
  - To study these issues in PNH, 133 patients with PNH (N=33), AA (N=33), and AA/PNH (N=67) were sequenced using deep targeted NGS covering all exons of the PIG-A gene.
  - Traditionally, PNH has been considered a monoclonal disease in which a PIG-A mutant HSC clone can outcompete normal hematopoiesis.
  - Results from the study indicate that initially, an environment that "favors" a state of GPI-anchor deficiency allows multiple clones to evolve. Over time, the most "fit" PNH HSC clones dominate, resulting in monoclonality.
  - While immune selection may provide initial conditions favorable for oligoclonal PNH evolution, once pressure is relieved, intrinsic factors can lead to PNH monoclonality.

**Questions?**