Paroxysmal Nocturnal Hemoglobinuria:

Understanding the Diagnosis, Complications and Treatment Options

Iberia Romina Sosa, MD, PhD
Assistant Professor of Medicine
Baylor College of Medicine
April 21, 2018

Paroxysmal Nocturnal Hemoglobinuria

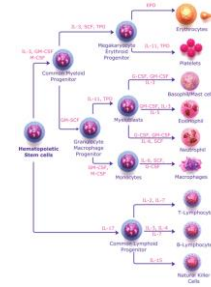
- PNH was first reported in the medical literature in the latter half of the 19th century.
- It was so named because of the mistaken belief that hemolysis (red blood cell break down) and subsequent hemoglobinuria (free hemoglobin in urine) occurred:
  - Intermittent episodes (paroxysmal)
  - Greater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

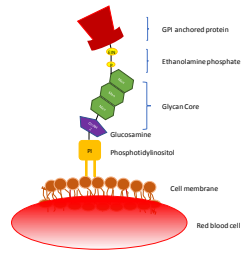
PNH Stem Cell

- 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

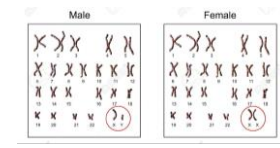


Disease Mechanism

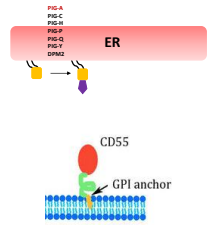
- The disease begins with the expansion of the hematopoietic stem cell that has severe deficiency or absence of GPI—a glycolipid moiety that anchors >150 different proteins.
- GPI deficiency is the result of a somatic mutation in PIG-A gene
  - Phosphotidylinositol glycan anchor biosynthesis, class A

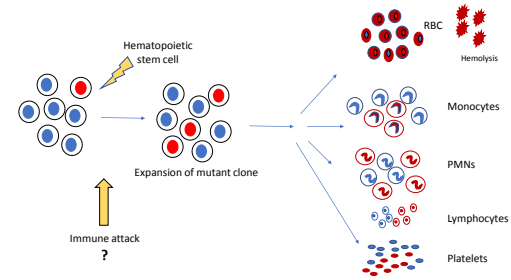


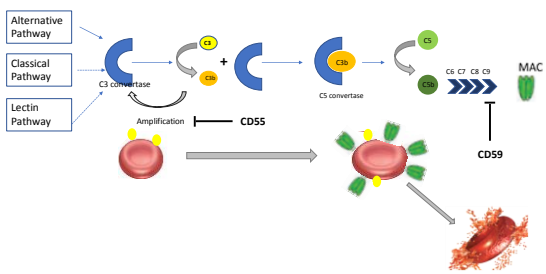
- PIG-A gene is found in the X chromosome
  - A single hit is enough to generate the PNH phenotype.
  - Males only have one X chromosome
  - Females undergo lyonization (chromosome inactivation).
- The mechanism for the mutations is unknown.
  - Typical mutagenic events such as radiation, chemotherapy, 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.





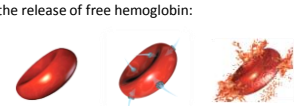


Classification of PNH

Classification	Example	Clinical Findings	Lab Findings	Bone marrow findings
Classic PNH		Dark/red urine Thrombotic complications of unusual sites	Elevated LDH, reticulocyte count and indirect bilirubin Low haptoglobin	Cellular marrow with erythroid hyperplasia No karyotypic abnormalities
PNH in the setting of another bone marrow disorder	PNH/Aplastic Anemia PNH/refractory anemia- MDS	Variable Hemolysis	Consistent with hemolysis	Abnormal bone marrow morphology Nonrandom karyotypic abnormalities
PNH-subclinical in the setting of another bone marrow disorder	PNH-sc/Aplastic Anemia	No hemolysis	Normal hemolysis labs Small populations of GPI-associated protein deficient cells	Abnormal bone marrow findings

Classic PNH

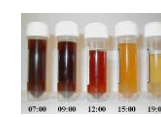
- Characterized by signs and symptoms of hemolysis or red blood cell breakdown.
- Symptoms directly attributed to anemia or the destruction of red blood cells:
  - Shortness of breath
  - Fatigue
- Symptoms indirectly associated to the release of free hemoglobin:
  - Dysphagia
  - Abdominal pain
  -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



Clinical Manifestations: Signs and Symptoms

- 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

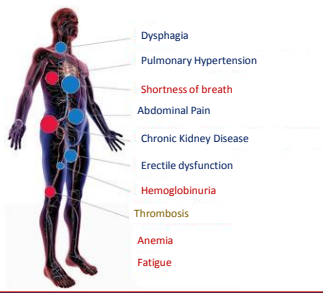


Laboratory Abnormalities

- 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

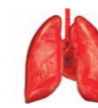
- Hypocellular, normocellular or hypercellular bone marrow often with erythroid hyperplasia
  - may see erythroid dysplasia
- Iron deficiency:
  - Low serum iron, low ferritin, incrased transferrin, absent bone marrow iron
- Creatinine and blood urea nitrogen may be increased (BUN) from acute and/or 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



Smooth Muscle Dystonia: Mechanism

Releases free hemoglobin

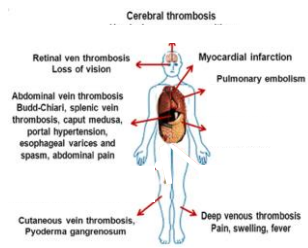
Nitric Oxide Scavenging:
Oxyhemoglobin reacts with NO to form methemoglobin and nitrate.



NO depletion impairs regulation of smooth muscle tone:
Vascular constriction
Pulmonary Hypertension
Shortness of Breath

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
- 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

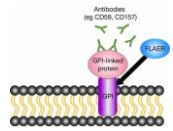
- No evidence to support routine imaging or laboratory testing.
  - Clinical suspicion!
- Rely on history and physical examination
- Baseline d-dimer may be useful to direct history taking to elicit symptoms of thrombosis
  - Neurologic symptoms, abdominal pain, leg swelling
  - Increased abdominal girth,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

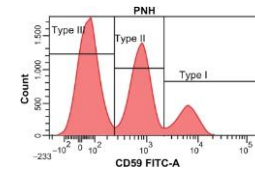
How do you diagnose PNH?

- Flow cytometry analysis using antibodies directed against GPI-associated proteins:
  - CD55, CD59
  - Quantification of at least two GPI-AP
- 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.

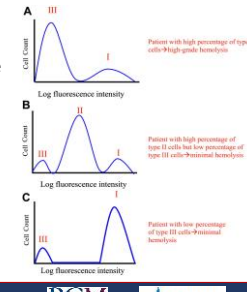
Charles Parker et al. Blood 2005;106:3699-3709

- 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.

PNH types	Clone Size in granulocytes
Classic PNH	40-99%
Acquired Aplastic Anemia	0.1-10%
MDS	<1%
Healthy Individuals	~0.002

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.

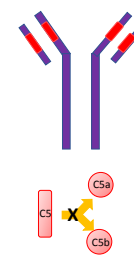
Therapy Options
Corticosteroids (hemolytic anemia)
Androgens (hemolytic anemia)
Transfusion
Splenectomy
Iron Replacement
Folate
Eculizumab
Bone marrow transplant

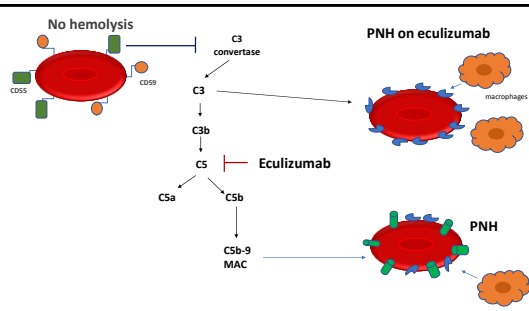
Treatment: Hemolytic Anemia

- 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
    - Steroids can reduce hemolysis but their limited efficacy and toxicity reduce make them less appealing
  - 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 eculuzimab for use in PNH based on its efficacy in two phase 3 clinical trials:
- Eculuzimab is effective in reducing intravascular hemolysis
  - Does not stop extravascular hemolysis
  - Does not treat bone marrow failure





TRIUMPH

- **T**ransfusion **R**eduction efficacy and safety clinical **I**nvestigation **U**sing eculizu**M**ab 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

SHEPHERD

- **S**afety in **H**a**E**molytic **PN**H patients treated with **E**culizumab: a multi center open label **R**esearch **D**esign
- 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

- 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.

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.

- Breakthrough intravascular hemolysis and a return of PNH symptoms occur in less than 2% of PNH patients on eculizumab
  - May occur a day or two before the next scheduled dose.
  - Spike in LDH
- If this occurs on regular basis:
  - Change the interval dosing to 12-13 days OR
  - Increase the dose to 1200 mg every 14 days
- Increased complement activation (viral infections) can result in breakthrough hemolysis
  - NO need to change the dosing if this is what precipitated the increase in hemolysis

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
- *Neisserial* sepsis
  - 0.5% yearly risk of Neisseria sepsis even after vaccination.
  - Revaccination against N. *meningitidis* every 3 to 5 years
- A major barrier to the use of eculizumab is cost:
  - Several hundred thousand dollars/year
- Continuous treatment is required to suppress intravascular hemolysis and/or thrombosis
- Patients unable to access this medication must be managed symptomatically

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.

Hall et al. Blood 2003: 102: 3587-3591.

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 eculuzimab is not available.
  - Failure to respond to eculuzimab due the heterozygous mutation c.2654G→A mutations in C5
  - Failure of eculuzimab 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

Thrombosis in Non-Hemolytic Paroxysmal Nocturnal Haemogloinuria

Morag Griffin, Talha Munir, Peter Hillmen, Louise Arnold, Nicola Copeland, Kathryn Riley, 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 also 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, Bhumika J. Patel, Taha 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?