

PNH 101

The basics of PNH

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What I want to do today...

- Give an update on PNH...
 - What is PNH?
 - What are the symptoms?
 - What causes PNH?
 - How is PNH diagnosed?
 - Current treatment options
 - Where are we heading
- Enter a dialogue about PNH...
 - What are your concerns?
 - What do you want to know about?



What is PNH?

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



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

What is PNH?

- A rare and unusual acquired hematologic disorder characterized by
 - Intravascular hemolysis (breaking of red cells)
 - Bone marrow failure (low blood counts)
 - Thrombosis (blood clots)
- There is a great deal of heterogeneity in the clinical presentation and course in patients with PNH

Signs & Symptoms of PNH

- Episodic dark urine (hematuria)
- Anemia
- Fatigue
- Abdominal pain
- Esophageal spasms (heartburn)
- Impotence
- Low blood counts (cytopenias)
- Blood clots

Signs & Symptoms of PNH

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

Fatigue in PNH

- Is very, very common
- May be out of proportion to the degree of anemia
- Gets worse with flares in hemoglobinuria
- Is probably related in part to complement activation and other inflammatory proteins
- Can markedly affect quality of life

What is the Extent of Fatigue and Impaired QoL in PNH?

- Burden of fatigue and QoL is extensive in a study of 29 PNH patients:
 - 96% complained of fatigue
 - 76% were forced to modify their daily activities to manage their PNH
 - 17% were unemployed due to PNH
 - >50% reported abdominal pain, headache and dyspnea
 - Patients commonly reported dysphagia (41%) and erectile dysfunction (47%)
 - Most symptoms rated as moderate to very severe, and a substantial majority reported distress associated with the symptoms

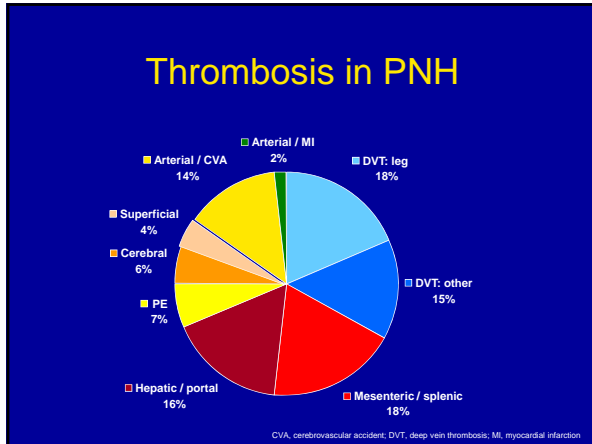
Meyers G et al. Blood (ASH Annual Meeting Abstracts), Nov 2007; 110: 3683.

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Thrombosis (Blood clots) in PNH

- Thrombosis is the most feared complication of PNH
- Occurs in 40% of patients
- Contributes to end organ damage-lungs, liver, kidney
- Leading cause of death
 - Accounts for 40–67% of deaths
 - First TE increases risk for death 5- to 10-fold
- Thrombosis can be the presenting symptom
- First thrombotic event can be fatal
- May be correlated with the size of the WBC clone
- May occur in funny locations

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What causes PNH?

- PNH is due to a change (mutation) in a single gene in a bone marrow “stem” cell.
 - What is a mutation?
 - What is a stem cell?

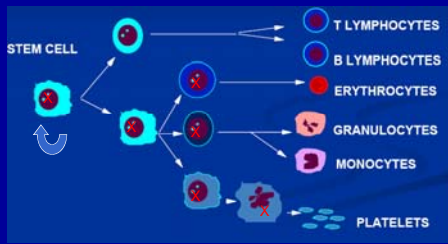
What is a mutation?

- A mutation is a “mistake” in a gene that arise when a cell divides and has to copy the DNA. This mistake is not corrected and is passed on to “daughter cells” and all subsequent cells.
- Mutations can cause:
 - No effect
 - Complete absence of the protein produced by that gene
 - An altered protein with decreased or different function

What is a stem cell?

- A stem cell has two properties
 - Can divide to produce daughter cells and more stem cells (self renewal) forever.
 - Can "differentiate" (mature) into many different types of cells.
- Many different types of stem cells
 - Embryonic
 - Tissue specific
 - Hematopoietic

Hematopoietic Stem Cells



In PNH, a mutation occurs in a single gene (PIG-A) in a single hematopoietic stem cell

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.
 - Sometimes the mutation leads to a partial decrease in the amount of anchor protein that is made and the cells have a partial deficiency (Type II cells)

What causes PNH?

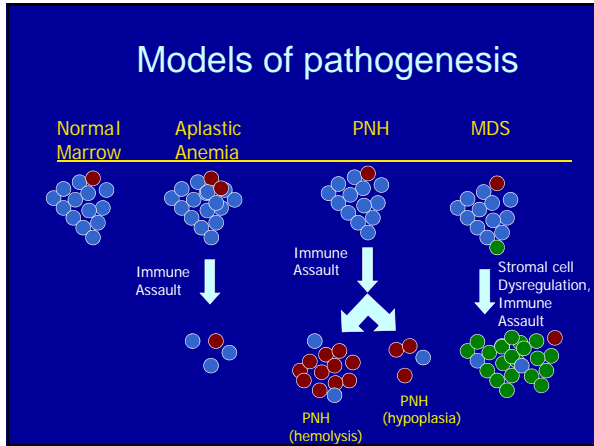
- The lack of the GPI anchor protein leads to a lack of many proteins on the surface of affected blood cells.
- In PNH, the major two proteins lacking on the surface of the red cells are CD59 and CD55.
- These proteins are important in protecting the red cells from complement.
 - What is complement? (three slides later)

What causes PNH? How do cells with a mutation take over the bone marrow?

- Normal people may carry cells in their bone marrow with a PIG-A mutation, usually at a very low level.
- In PNH, something allows the abnormal cells to become the dominant cells and become the major population in the marrow (anywhere from 1 to over 90% - referred to as the clone size).
 - This "something" may be related to aplastic anemia, a disease of poor production of the bone marrow

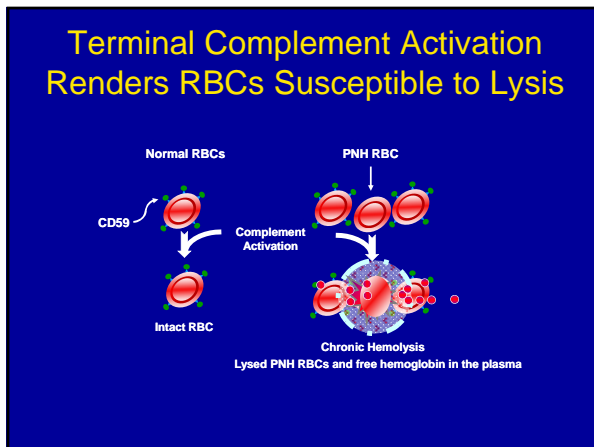
How is PNH related to aplastic anemia?

- Many patients with PNH have or will develop aplastic anemia, or have a history of having had aplastic anemia.
- Many PNH patients have evidence of poor production of cells by their bone marrow (Bone marrow failure) leading to low white cell counts or low platelet counts.
- The cause of aplastic anemia (immune assault?) may also play a role in the development of PNH.



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)



What happens when red cells lyse?

- 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 oxide?

- 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

What about thrombosis (blood clots) in PNH?

- Blood clots are a presenting sign in 10-20% of patients with PNH.
- Can occur in up to 40% of patients with PNH.
- Occur in unusual locations – veins of the liver (Budd-Chiari syndrome), spleen, brain, and skin.
- Associated with a very bad prognosis
- Cause of these blood clots is unknown – possibly related to complement activation.

The clinical picture of PNH

- Hemolysis due to complement activation
 - Anemia and fatigue
 - Hemoglobinuria, kidney damage
 - Nitric oxide trapping >> Esophageal spasm, abdominal pain, pulmonary hypertension, impotence, fatigue?
- Thrombosis – possibly due to complement activation
 - Unusual sites of blood clots
- Bone marrow failure
 - Decreased blood counts (cytopenias)

How is PNH diagnosed?

Average delay to diagnosis exceeds 3 years; may be greater than 10 years

- PNH continues to be primarily a clinical diagnosis, which can be confirmed by laboratory analyses
- Signs and symptoms are highly variable and may mirror other conditions
- Most common symptoms at presentation are not unique to PNH
 - Hemolytic anemia, often requiring transfusions
 - Fatigue
 - Dyspnea
 - Abdominal pain or dysphagia

The problem with diagnosing PNH – Educating the right people

When you hear hoofbeats, ...



When you don't find the horse,
learn how to spot the zebra

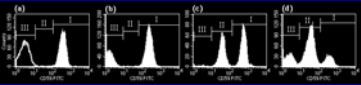


Methods of Historical Interest

- 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

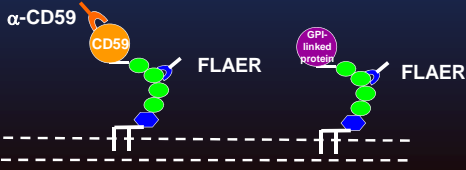
- Perform on peripheral blood
- Use monoclonal antibodies against GPI-anchored proteins, such as CD59 or CD55^{1,2}
- PNH blood cells (PNH clone) are cells missing GPI-anchored proteins



¹Parker, et al. *Blood*. 2005;106:3699-3709.
²Hall & Rosse. *Blood*. 1996;87:5332-5340.

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



How do we treat PNH?

- Hemolytic anemia
 - Iron, folic acid
 - Transfusion
 - Steroids
 - Eculizumab
- Thrombosis
 - Coumadin prophylaxis
 - Acute treatment with lytic agents (clot busters)
 - Anticoagulation therapy
 - Bone marrow transplantation
- Bone marrow failure
 - Stimulating agents such as erythropoietin
 - Immunosuppressive agents (ATG, cyclosporine A)
 - Bone marrow transplantation

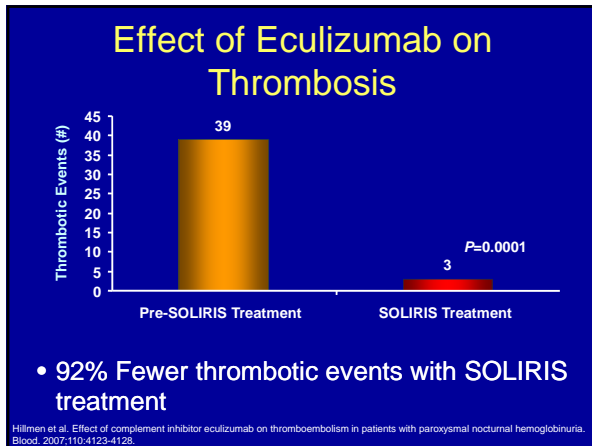
SOLIRIS™ (eculizumab)

SOLIRIS is a complement inhibitor indicated for the treatment of patients with PNH to reduce hemolysis

SOLIRIS is the first FDA approved therapy for PNH

What does Eculizumab do?

- Quickly and effectively blocks complement activation.
- Blocks hemolysis and related effects
- Stops hemoglobinuria
- Markedly reduces transfusion requirements
- Hemoglobin / hematocrit may not return to “normal”



- ### Possible long term effects of Eculizumab
- Improve kidney function
 - Improve hypertension
 - Prevent pulmonary hypertension
 - Increase survival

- ### What eculizumab does not do
- Probably does not help bone marrow failure (improve other low blood counts)
 - Completely correct anemia
 - Bone marrow failure (poor production)
 - Extravascular hemolysis
 - Teach you how to play the violin if you have never played before

Downside of Eculizumab treatment

- Increased risk of meningococcal infections
 - All patients must be vaccinated
 - All patients educated on signs and symptoms of meningitis and what to do
 - All patients given cards describing this
- Cost
- Inconvenience
 - Must be given intravenously every 12-14 days

Who should receive Eculizumab?

- PNH patients with symptomatic hemolytic anemia
- PNH patients with thrombosis

Where are we going?

- Improve current therapy
 - Oral eculizumab
 - Increase treatment intervals
 - Understand extravascular hemolysis
- Find other ways to inhibit complement
- Understand how PNH cells take over the bone marrow so we can reverse this process (Restore normal stem cells)
- Improve our understanding of what leads to blood clots (and can prevent blood clots)
- Gene therapy
- Stem cell transplants

