Paroxysmal Nocturnal Hemoglobinuria

Current Thinking On the Disease Diagnosis and Treatment

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Objectives

- Try to answer some of the frequently asked questions about:
  - The cause of the PNH
  - The clinical presentation of PNH
  - Diagnosing PNH
  - The complications of PNH
  - New treatments for PNH
A CASE
OF
INTERMITTENT HÆMATINURIA,
WITH REMARKS.

By WILLIAM W. GULL, M.D.

Pt. J.G. 33 y.o. Leather Worker

Infected Lymph gland

February

March

Indigo
Dark Mahogany
Mahogany
Red
Amber
What is PNH?

- A disorder of blood affecting all the cells which come the bone marrow.
- The disease is quite rare, only 10,000 patients in the US and Europe.
- There is no ethnic preference for the disorder.
- It may present early or late in life.
- The manifestations may be “classic” or obscure.

What is PNH?

- PNH is due to a mutation in a gene in a blood stem cell.
- The gene is called the PIG-A gene and is located on the X chromosome.
- In most cases of PNH, the change in the gene (mutation) is acquired, not something you are born with. When and why is unknown.
- The gene contains the genetic information for the GPI anchors which link proteins to the cell membrane.
What is PNH?

- A mutation is a “mistake” or a “change” in the gene that arises during copying and is not corrected.
- When the cell divides, the mutation is transmitted to daughter cells.
- The effect of a mutation:
  - None
  - An altered protein (sickle hemoglobin)
  - No protein is produced as in PNH, hemophilia etc.

What is PNH?

Evolution of PNH in Marrow

![Diagram showing normal and abnormal clones evolving in marrow](image)
What is PNH?

- Makes a protein
- Copies itself for a new cell
- When the cell divides, the new cells then contain the new genetic information

GPI-AP Biosynthesis: Involves 10 Steps and >20 Genes
What is PNH?

As a result of the PIG-A mutation, there is little of no GPI anchor produced.

- PNH II cells - mild reduction
- PNH III cells - severely reduced.
- When the anchor is reduced, certain proteins can’t attach to the cells.
- The most important proteins for PNH are CD 59, CD55.

What is PNH?

- Many normal people have very small numbers (perhaps 6 per 1,000,000 bone marrow cells)

- In PNH, the abnormal cells have an advantage and become a major population in the marrow and blood (anywhere from 1% to over 90%)
  - This may be a result of change in the immune system – inability to recognize something foreign.
  - Or it may be related to aplastic anemia, a disease of poor production of blood from the marrow
PNH Clinical Features
Aplastic Anemia

- Some PNH patients have aplastic anemia or a history of aplastic anemia
- Many PNH patients have evidence of a bone marrow that doesn’t work well or well enough to maintain normal blood counts
- Therefore, whatever causes aplastic anemia (immune suppression or dysregulation or damage to the stem cells) may allow PNH to develop

What is PNH?
Complement

- Complement is a group of blood proteins that act together to help the body get rid of microbiological invaders
  - One of the ways it does this is by penetrating the membrane (outside surface) of the invading bacteria or viruses.
  - When this happens to PNH blood cells, the cells are destroyed.
What is PNH?

Complement circulates in an inactive form

- It is activated spontaneously and by a variety of events
  - It is normally activated more at night
  - It is more active with infections, trauma, vaccinations, surgery, immune complexes, autoimmune diseases

What is PNH?

Complement activity is regulated by proteins in the blood and on the membranes of the cell.

Proteins on the cell surface interfere with complement to prevent breakdown (lysis) of the cell membrane

- The most important of these is CD59, which is missing on the abnormal cells of PNH
- For this reason, PNH red cells are extremely sensitive to very small amounts of activated complement
Absence of CD59 Allows Terminal Complement Complex Formation

Adapted from Cellular and Molecular Immunology AK Abbas, AH Litchman and JS Pober, 3rd Edition. 1991 WB Saunders; Philadelphia.
What is PNH?

- Complement successfully attacks the red cells and they break up (hemolysis)
  - This releases hemoglobin (the red pigment in red cells) into the plasma
  - Causes anemia
  - Pieces of the membrane come off
- The white cells release granule contents and change to express other proteins
- The platelets form vesicles (membrane blisters) and activate

Normal red blood cells are protected from complement attack by a shield of terminal complement inhibitors.

Without this protective complement inhibitor shield, PNH red blood cells are destroyed.

Complement activation
What is PNH?
Clinical Features

- Some of the hemoglobin passes through the kidneys and into the urine, causing red to dark brown urine (hemoglobinuria)
  - This causes a loss of iron from the body
  - In the long run, this may damage the kidney
- Free hemoglobin binds nitric oxide causing vascular and smooth muscle spasm
- Causes inflammation

What is PNH
Clinical aspects

- Vascular (arterial constriction, HBP)
- Pulmonary artery pressure increase (PHTN)
- Spasm of the esophagus
- Abdominal pain
- Erectile dysfunction
- Other symptoms such as “fatigue”
- Platelets are more “reactive”
What is PNH?
Clinical Features

- **WBC**: Granulocytes - release content stimulating inflammation
  - Monocytes - activate expressing TF which leads to blood clots. TF-Microvesicles
- **Platelets** become “activated”
  - They stick together and form clumps
  - The membrane changes, allowing them to bind to monocytes
  - Pieces of the membrane come off (microvesicles)

What is PNH?
Clinical Features

- **Hemolytic anemia due to complement activation**
  - Hemoglobinuria and, eventually, kidney damage
  - Anemia to a variable degree
  - Effects of NO depletion- HBP, smooth muscle dystonia, reduced blood flow to the kidney and lungs
- **Impaired bone marrow function**
What is PNH?
Clinical Features

- Thrombosis (Blood clots)
  - Often in unusual places (liver veins, abdominal veins, cerebral veins, dermal veins)
- Fatigue – overwhelming, poor correlation to level of hemoglobin
  - inflammation
  - anemia
  - PHTN

Thrombosis in PNH Pathophysiology

TF = Tissue factor.
What is PNH
Diagnosis of PNH

- Historical test – Sucrose hemolysis, Hamm’s test no longer used
- **Flow cytometry on peripheral blood is the gold standard for diagnosing PNH**¹
- Both granulocytes and erythrocytes should be tested²
  - Erythrocytes alone are not sufficient due to hemolysis and dilution effect of transfusions
- Multiple monoclonal antibodies against GPI-anchored proteins (such as CD59 or CD55) are used¹²
- PNH blood cells (PNH clone) are cells that are missing GPI-anchored proteins


Historical Management Options for PNH

Generally conservative, supportive, and dependent on symptom severity¹²

- Transfusions
- Anticoagulants
- Supplements
  - Folic acid
  - Iron
  - Erythropoiesis stimulating agents
- Steroids/androgen hormones
- Allogeneic bone marrow transplant
  (limited eligibility)
What is Soliris®?

- Monoclonal antibody (protein) that blocks complement at C5 preventing the formation of the terminal complement complex
- Quickly and markedly reduces hemolysis
  - Stops hemoglobinuria
  - Increases hemoglobin level
    - Reduces transfusions
    - Hematocrit may not be quite normal

SOLIRIS Blocks Terminal Complement

- SOLIRIS binds with high affinity to C5
- Terminal complement activity is blocked
- Proximal functions of complement remain intact
  - Weak anaphylatoxin
  - Immune complex and apoptotic body clearance
  - Microbial opsonization

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Reduction in LDH During Soliris® Treatment in TRIUMPH and SHEPHERD

PI: All patients sustained a reduction in intravascular hemolysis over a total SOLIRIS exposure time ranging from 10 to 54 months.

TRIUMPH placebo patients switched to SOLIRIS after week 26. All TRIUMPH patients entered the long term extension study.

D.T., urine score 2 weeks before & after Eculizumab
Effect of Soliris® on Ability to Maintain a Good Hemoglobin

Effect of Soliris® on Transfusion in PNH
What is the effect of Soliris® in PNH

- Stops the symptoms associated with hemolysis
  - “Fatigue”
  - Esophageal and abdominal spasm
  - Erectile dysfunction
  - Improves sense of well being
  - Reduced the need for transfusion

- Appears to reduce thrombosis (blood clots)
Effect of Eculizumab on the blood clots in PNH

Equalized Patient Years (195)

Patients on Antithrombotics n=103 (P < 0.000000001)

92% reduction in TE events with Eculizumab

91% reduction in TE event rate with Eculizumab

Effect of Eculizumab on markers of hemostatic activation

Eculizumab treatment:

- (Weitz, IC et al 2008) All but 2/11 patients were activated pre-treatment both were receiving prophylactic anticoagulation
  - There was a statistically significant decrease in LDH (p<.0001), D-D (p=0.0057), TAT (p=0.0138), IL-6 (p=0.0362) during the 4 week induction phase of treatment (D29).
  - All decreases in D-D, TAT, IL-6 and LDH were sustained in the maintenance phase of treatment (D29-90). There was no correlation with LDH (DD =0.143, TAT p=0.188)

- (Helley, D et al 2009) pre vs week 5, 11
  - There was a significant decrease in plasma measures of coagulation activation (prothrombin fragment 1+2, P=0.012, and D dimers, P=0.01), and a lower consecutive fibrinolysis (tissue type plasminogen activator, P=0.0005, and plasmin antiplasmin complexes, P=0.0002).
  - Decreased plasma measures of endothelial cell activation (soluble vascular cell adhesion molecule, P<0.0001, and von Willebrand antigen, P=0.0047) and
What is the Effect of Soliris®?

Improves kidney function
- reduced hemoglobinuria and iron deposition
- Reduced thrombosis

Improves hypertension
May in part be due to availability of nitric oxide

Effect of Eculizumab On Overall Survival in PNH

- Pre-Eculizumab
  - Actuarial Survival From Time of Diagnosis in 80 Patients With PNH

- Post-Eculizumab
  - N=79

Side Effects of Soliris® Treatment

- Susceptibility to sepsis by meningococcal organism
  - All patients must be vaccinated at least 2 weeks before starting Soliris
  - All patients must know to seek medical help at once when fever happens
  - All patients must carry cards describing this complication

- Headache – first week or 2

Cost

Inconvenience

- Must be given every 12-14 days by vein

What Soliris® Cannot Do

- Does not appear to improve impaired bone marrow function
  - Low white count or low platelet count may persist in some patients, especially if it is due to aplastic anemia
  - Other treatments may be indicated
    - Bone marrow transplantation
    - ATG and other immunosuppressives
When is Soliris Ineffective or Less Effective

- Patient has been incorrectly diagnosed with PNH.
- Patient has a very small PNH clone (less than 10%)  
  bone marrow failure - AA
- Breakthrough – infections, increased clearance, delayed dosing
- Extravascular hemolysis

Who Should Get Soliris®?

In all cases, the decision should be made by a physician that understand PNH, its complications and its treatment in conjunction with the patient, whose life is affected by the disorder.
Which PNH Patients are Candidates for Soliris?

- Patients with hemolysis (LDH ↑)
- Patients with large CD 59 deficient clones- RBC, WBC
- Patients with hemolysis with evidence of renal impairment  GRF<90, proteinuria etc
- Patients with history of thrombosis
- Patients with hemolysis and elevated Ddimers
- Patients with hemolysis with fatigue
- Patients with hemolysis and transfusion dependence
- Patients prior to ?BMT, surgery, ???pregnancy
Effect of eculizumab on markers of haemostatic activation and inflammation

- LDH: $p < 0.0001$
- D-dimer: $p = 0.0057$
- TAT: $p = 0.0138$
- IL-6: $p = 0.0362$